

Towards stochastic simulation of crop yield: a case study of fruit set in sweet pepper

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Abstract

Crop growth simulation models are widely used in research and education, and their use in commercial practice is increasing. Usually these models are deterministic: one set of input values always gives the same output of the model. In reality, however, variation exists between plants of the same crop. A simulation model taking this variation into account is therefore more realistic. The aim of this thesis is to introduce a stochastic component into a dynamic crop simulation model. As case study, fruit set in sweet pepper was used, because large variation in fruit set between the plants exists. Competition with fast growing fruits causes abortion of flowers and young fruits, which results in periods with high and low fruit set, and consequently periods of high and low fruit yield. A literature review showed that most factors influencing fruit abortion can be expressed in the terms source and sink strength. Source strength is the supply of assimilates; a higher source strength increases fruit set. Source strength takes into account leaf area, radiation, and CO₂ level and temperature. Sink strength is the demand for assimilates of the fruits and vegetative parts. It is quantified by the potential growth rate, i.e. the growth rate under non-limiting assimilate supply. Assimilate demand of the fruits depends on their number, age, and cultivar. If the total fruit sink strength of a plant is low, fruit set is high. Vulnerable for abortion were very small buds, buds close to anthesis and flowers and young fruits up to 14 days after anthesis. An experiment with six *Capsicum* cultivars with fruit sizes ranging between 20 and 205g fresh weight showed that variation in weekly fruit yield is highly correlated with variation in weekly fruit set. Fruit yield patterns resembled fruit set patterns, with a lag time being equal to the average fruit growth duration. Further investigation showed that the cultivars not only differed in sink strength of the individual fruits, but also that the source-sink ratio above which fruit set occurred was higher in cultivars with larger fruits. In the second half of the thesis, flower and fruit abortion was modelled. Survival analysis was used as the method to derive the abortion function. Source and sink strength were used as the factors influencing abortion. Their effect on the probability of abortion per day was non-linear: at high values of source and sink strength an increase did not further decrease or increase the probability of abortion, respectively. Flowers on the side shoots turned out to have a higher probability of abortion than flowers on the main shoot. Most flowers and young fruits aborted around 100°Cd after anthesis. The obtained function was used in a crop simulation model for sweet pepper. After calibration the model was able to simulate the observed fruit set pattern, although fruit abortion was not properly simulated when low source strength was combined with high sink strength. Validation with three independent data sets gave reasonable to good results. Survival analysis proved to be a good method for introducing stochasticity in crop simulation models. A case study with constant source strength showed asynchronisation of fruit set between the plants, indicating that fluctuations in source strength are an important factor causing synchronisation between individual plants.

Key words: fruit abortion, fruit set, *Capsicum*, survival analysis, crop simulation model, source strength, sink strength, temperature.

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Chapter 1

General introduction

In this first chapter the background is given for the research presented in this thesis. The first part of this chapter focuses on the general principles of crop simulation models, and how fruit set is simulated in these models. In particular, attention is paid to models with a stochastic component. The second part focuses on variation in fruit set, especially on variation in fruit set in sweet pepper. Both parts lead to the aim of the thesis, given at the end of this chapter.

Crop growth simulation models

General description of simulated processes

Crop growth simulation models are widely used in research. One of their functions is to help researchers answer questions which might otherwise require extensive experiments (Gary *et al.*, 1998). Growers are also becoming more and more interested in the use of crop growth simulation models, for example for making decisions regarding crop management based on model calculations (Heuvelink and Kierkels, 2007).

Leaf area is an important variable in crop growth models, as the leaves absorb radiation and CO₂ resulting in photosynthesis, which is the basis for crop growth. Leaf area expansion depends on temperature and/or assimilate supply (Goudriaan and Van Laar, 1994). Based on leaf area, radiation levels, temperature and in some cases CO₂ concentration, the daily growth of the whole crop is calculated. The growth of the individual organs or groups of similar organs can be calculated in different ways. In crops with determinate growth (where growth of the plants stops when the floral reproductive structure has been formed), the daily growth of the different organs is often calculated as a fraction of the daily growth of the total plant. This fraction varies with the developmental stage of the crop (Goudriaan and Van Laar, 1994). In crops with indeterminate growth (where new flowers are formed continuously) a more dynamic function for distribution of dry matter between the different organs is desirable. This is usually done based on the concept of sink strengths (Marcelis, 1996). The sink strength is the demand for assimilates which is often quantified by the potential growth rate. The total sink strength of the plant is the sum of the sink strengths of individual organs. The daily growth of one organ is calculated by its share in the total sink strength multiplied by the daily growth of the plant.

Simulation of fruit abortion

Plants with an indeterminate growth pattern can show strong fluctuations in fruit abortion over time. This makes it difficult to model abortion of flowers and fruit. Consequently, the abortion process is a less well-developed part of simulation models for these crops. Abortion of flowers and fruit is often modelled based on the balance between supply and demand of assimilates, the so-called source-sink ratio. In the tomato growth model TOMGRO (Bertin and Gary, 1993; Heuvelink and Bertin, 1994), fruit abortion increases

linearly with decreasing source-sink ratio from 0% at a source-sink ratio of 0.42 up to 70% abortion at a source-sink ratio of zero (Bertin and Gary, 1993). Marcelis (1994) used the source-sink ratio and temperature to determine the number of young non-aborting fruit in cucumber. Schepers *et al.* (2006) simulated the abortion of sweet pepper fruit based on their fruit weight and the assimilate supply: above a certain threshold, a fruit would not abort any more. Buwalda *et al.* (2006) simulated fruit set in sweet pepper based on an empirical function where the difference between source and sink strength determined the number of newly set fruit. In the crop simulation model INTKAM, the number of flowers and young fruits a plant can sustain depends linearly on the gross assimilation of the plant, the total fruit sink strength and the temperature (Marcelis *et al.*, 2006). If the number of young flowers and fruits is higher than the plants can sustain, the youngest ones abort.

Simulation with variation

Even when crop models simulate abortion of individual organs, it is assumed that each organ follows the same deterministic rule in relation to the factors influencing abortion. This is usually the case for all modelled processes in crop growth and development for all types of crops. Models simulate an average plant or crop resulting in a single output value for each variable.

Only a few examples of crop growth models simulating variation in output variables exist. Pearson *et al.* (1996) developed a stochastic model for truss appearance in tomato. The truss appearance rate was assumed to be normally distributed; the mean rate depended on temperature and the standard deviation was kept constant. This led to plant-to-plant variation in the set of trusses. Agostini *et al.* (1999) simulated the number of flowers and timing of flowering in kiwi stochastically. The number of buds was determined by a probability distribution and depended on vine length. The probability and timing of bud break were determined by position and orientation of the bud. The disadvantage of using such a predetermined probability density distribution is that it does not allow for feedback of the (environmental) circumstances on the probability.

An alternative to achieve variation in simulation output is to add variability to the outcome of deterministic models, to be applied after the simulation. Benjamin *et al.* (1999) established relationships between mean weight and the variation in mean weight of different size categories, which could be used to calculate the variation in weight when only the mean weight was simulated. In this case, the variation in model output is not an intrinsic part of the simulation model. Variation in model outcome can also be simulated by conducting large numbers of simulation runs where different parameter combinations (within predefined limits) were used as input (Monte Carlo simulations; e.g. Bouman and Jansen, 1993) or with parameter values drawn from a theoretical distribution (e.g. Scholten and Van der Tol, 1994). However, this can result in unrealistic combinations of parameter values used in the model simulations.

The most sophisticated example of a model containing a probability function is presented by Lieth *et al.* (1986). These authors developed a simulation model for abscission of cotton bolls. The probability of abscission of the cotton bolls was calculated on the basis of their age. If the source-sink ratio was lower than one, the probability of abortion increased linearly with the decreasing source-sink ratio.

We can conclude that the field of stochastic simulation in crop science is largely an unexplored area. Adding a stochastic component to crop simulation models improves their

quality by predicting both average values and confidence intervals for the model output. The choice of the developmental process which should be simulated with variation depends on the crop. In the stochastic model the influence of the circumstances on the probability that a certain event will happen should be incorporated explicitly.

Survival analysis techniques analyse the timing of events and relate this to factors affecting this timing (Kleinbaum and Klein, 2005). The analysis yields a function which calculates the rate of the occurrence of the event per unit of time depending on certain factors. Survival analysis is therefore a promising technique to obtain a function which can be used to introduce stochasticity into a dynamic simulation model. Survival analysis has certain attributes which make its use very attractive. One of the attributes is that it can handle censored data where the event of interest has not yet occurred. This happens when an experiment has ended before all subjects have experienced the event or when a subject has, for instance, been removed. Another attribute is that the data also does not need to be normally distributed. Furthermore, survival analysis is very flexible in the way it handles factors influencing the event. These factors can be fixed, i.e. have a constant value during the full survival time of a subject, or their values can change during the survival time of a subject, the so-called time-dependent factors. In addition, random effects such as flowers growing on the same plant can be taken into account. Survival analysis originates from the medical science, but its use is increasing in the field of biology. Examples are the analysis of behavioural data (Vos *et al.*, 1998), seed germination (Vange *et al.*, 2004) and leaf abscission (Dungan *et al.*, 2003).

Variation in fruit set

Despite growing under the same circumstances, variations exist between plants within a crop, e.g. plants have different numbers of fruit and/or a different pattern in fruit set. In pea, the place of the first reproductive organ could vary two or three nodes (Guilioni *et al.*, 1997; a node is a point on the plant where a leaf or flower is attached). This leads to different numbers of reproductive organs. Pearson *et al.* (1996) reported the number of trusses in tomato plants varying between 25 and 35. In crops with an ear or composed flower, not all florets set. In sunflower, florets in the outer radius were more likely to set than florets in the centre of the flower (Alkio *et al.*, 2003). In maize, there was a considerable variation in the number of kernels per plant related to dominating and dominated plants (Pagano *et al.*, 2007).

The case of sweet pepper

In sweet pepper, variations exist in the position of the fruit on the plant between individual plants, although over a number of plants an average pattern of nodes with fruit and nodes without fruit can be observed (Fig. 1.1). This leads to a major problem occurring in sweet pepper production: yield fluctuations. Periods with high fruit yield alternate with periods of low fruit yield (Fig. 1.2). The fluctuations in fruit yield cause problems at several points in the production chain. The grower experiences fluctuations in labour requirement due to the different numbers of harvestable fruit. All growers in the country experience fluctuations in fruit yield and the peaks in fruit yield appear more or less simultaneously. This causes problems for the other parties in the production chain such as irregular supply to the market and fluctuating prices as a result. Reducing the fluctuations in fruit yield is therefore of

great economic importance. Growers and researchers have tried several possible solutions (Wien *et al.*, 1993; Heuvelink *et al.*, 2004; Buwalda *et al.*, 2009), but these have not been very successful, or were not able to achieve regular fruit set during the whole cultivation.

Fluctuations in yield are caused by fluctuations in flower and fruit abortion. Levels of flower and fruit abortion are substantial in sweet pepper: only 20-30 % of the flowers result in a harvestable fruit (Marcelis *et al.*, 2004). One of the main causes for the high levels of abortion is the competition between flowers and fruit on the same plant. This is caused by the way in which sweet pepper plants are grown. In the Netherlands sweet peppers are grown in glasshouses nearly all year round. Seeds are sown in October and young plants are planted in the glasshouse at the end of November or the beginning of December. The first flowers reach anthesis in January.

Sweet pepper plants have a dichotomous growth: an internode ends with a leaf and a flower, and the stem splits. Each new stem again produces a leaf and a flower after an internode, and splits. The weaker branch of each branching is pruned after the first leaf and flower, and the stronger one continues to grow. This type of pruning results in plants with long stems which continually produce new flowers. In commercial practice two, three or four main stems per plant are maintained supported by a training system. On one stem reproductive organs of various ages (buds, flowers and fruit) are present which compete for the same resources. This competition causes abortion of buds, flowers and young fruits. Apart from the competition between reproductive organs of various ages several other factors are also known to influence abortion levels. In short, abortion of organs can be induced by low supply of assimilates (source strength), high competition from other organs (sink strength) (Marcelis *et al.*, 2004), high temperatures (Aloni *et al.*, 1994), and shortage of water and nutrients (Guilioni *et al.*, 2003). Hormonal dominance of one organ over the other also plays a role (Bangerth, 1989). Qualitative effects of the factors influencing abortion are known, but the most researchers use simple linear relationships for quantification.

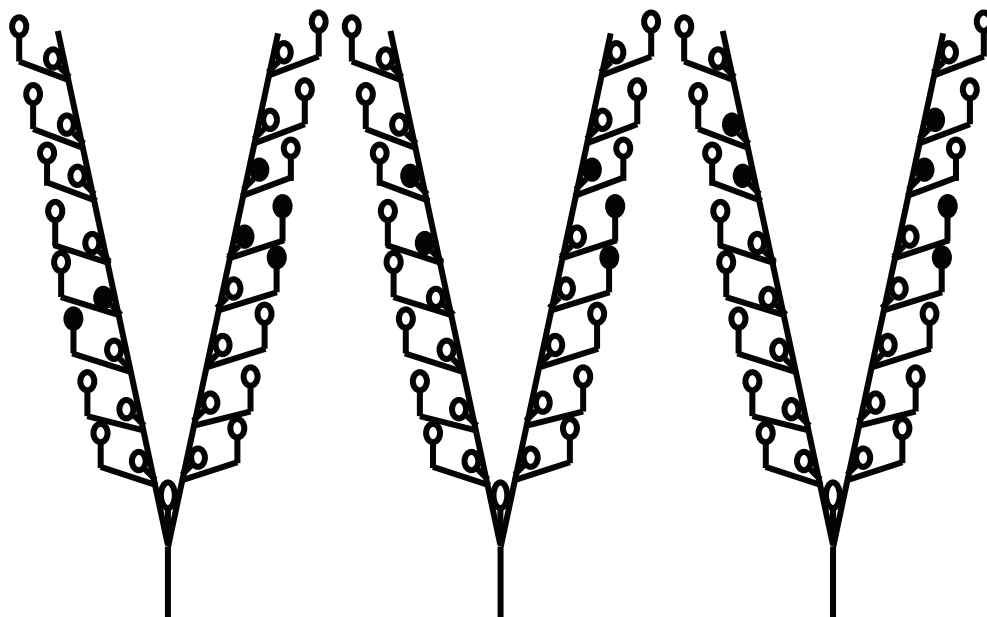


Fig. 1.1 Example of variation in fruit set in plants grown under equal conditions. Open bolls represent aborted fruit, closed bolls set fruit. Variation exists in the number of set fruit as well as in the position of the fruit on the plant.

Aim of the thesis

Developing new crop models with a stochastic component or incorporating a stochastic component in existing crop models would mean a major improvement in crop growth simulation. As crops are grown for their harvestable product, crop yield (kg ha^{-1} or g m^{-2}) and, if applicable, its components (number of harvested fruit, individual fruit weight), are important output variables. The aim of this thesis is to introduce stochasticity in an existing crop model in which the yield is the principle output variable simulated with variation.

Sweet pepper is used as an example. Considerable variation in yield over time exists, as well as variation between plants. The variation in crop yield is caused by variation in fruit abortion. The variation in flower and fruit abortion between the plants makes simulation of abortion using a deterministic simulation model unrealistic. A probability function which determines for each fruit its probability of abortion is a much more realistic option. The probability of abortion should depend on factors influencing abortion such as the number of competing fruit. The probability function is obtained with survival analysis.

The introduction of a stochastic abortion time derived from survival analysis into a dynamic crop growth model is an innovative new approach in crop growth modelling. This results in more realistic simulation of flower and fruit abortion, because mean and variance of fruit set are simulated. The crop growth model into which the probability function is built is INTKAM. This model has been validated earlier for total biomass and total fruit production of sweet pepper (Marcelis *et al.*, 2006).

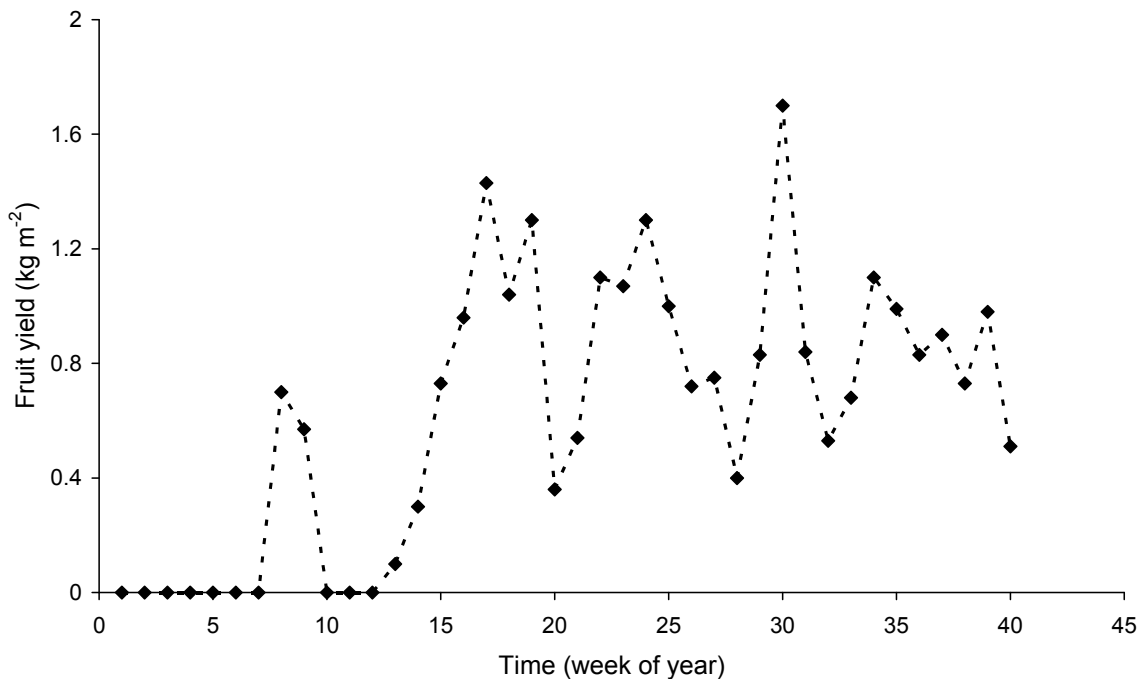


Fig. 1.2 Typical pattern of yield fluctuations in a commercial greenhouse.

Thesis outline

In order to be able to establish a relationship between the abortion rate and factors influencing this abortion rate it is necessary to know which factors influence abortion. The first part of the thesis (Chapters 2 – 5) focuses on factors influencing abortion. Chapter 2 is a literature review of factors influencing abortion, processes occurring during abortion and possible solutions to decrease the level of flower and fruit abortion or to diminish the fluctuations in flower abortion. The sink strength, which is important for simulation of dry matter partitioning and flower and fruit abortion, is derived from the measurements of fruit growth. Chapter 3 shows how fruit growth functions are obtained. Fruit growth functions are compared between different cultivars and temperatures. Chapter 4 compares fruit set and yield fluctuations among cultivars with different fruit sizes. In Chapter 5, the fluctuations in fruit set from Chapter 4 are analysed in detail. Differences in fruit set patterns between cultivars are explained on the basis of differences in underlying physiological characteristics (source and sink strength).

Once the factors influencing abortion of flowers and fruit are established, the effects of these factors need to be quantified. This is done in the second part of the thesis. In Chapter 6, survival analysis is used to analyse data of flower and fruit abortion and to quantify the most important factors influencing abortion. This yields a function which calculates the probability of abortion per day. In Chapter 7, this function is incorporated in the simulation model INTKAM. The function is calibrated and tested on independent data sets. Special attention is paid to the variation in simulation output.

Chapter 8 contains the general discussion, where the results of all chapters are combined, discussed and put into a broader context.

Appendix Chapter 1

Definitions of source and sink strength

Source and sink strength are central terms in the simulation of flower and fruit abortion and dry matter partitioning in many simulation models. The definitions of source and sink strength as used in the present thesis are given below.

Source strength

Source strength is the supply of assimilates on a certain day, originating from the photosynthesis and available for the growth of organs. Source strength is defined as the gross assimilation minus the maintenance respiration. Depending on the model used, it is expressed as $\text{g CH}_2\text{O plant}^{-1} \text{ d}^{-1}$ or as $\text{g dm plant}^{-1} \text{ d}^{-1}$. Maintenance respiration is subtracted because maintenance of existing organs has priority over growth of organs. In this thesis, source strength is not measured directly, but obtained from model simulations.

Sink strength

Sink strength is the demand for assimilates from an organ or a group of organs. It stands for the competitive ability of an organ to attract assimilates. Sink strength is quantified by the potential growth rate. An organ grows potentially when it grows under non-limiting assimilate supply. Non-limiting assimilate supply is created by growing the plants under high irradiance, low planting density and/or reduction of the number of competing fruits. The latter is applied to obtain the potential growth of fruits. In Chapter 3 it is described how the fruit growth curves, which are the basis for sink strength, are obtained. Sink strength of the vegetative part is more difficult to obtain. De Koning (1994) advocated that obtaining the vegetative sink strength via growth at high assimilate supply can lead to overestimation due to storage of excess assimilates (Strack *et al.*, 1979) or underestimation due to leaf growth reduction at high source-sink ratio's (e.g. Short-Leaf-Syndrome; Nederhoff *et al.*, 1992). It is therefore estimated from measurements on dry matter partitioning between the fruits and the vegetative part, total dry matter production and total calculated sink strength of the fruits. Depending on the model used, sink strength of a fruit is expressed as $\text{g CH}_2\text{O fruit}^{-1} \text{d}^{-1}$ or as $\text{g dm fruit}^{-1} \text{d}^{-1}$, and of the vegetative part as $\text{g CH}_2\text{O plant}^{-1} \text{d}^{-1}$ or as $\text{g dm plant}^{-1} \text{d}^{-1}$.

Chapter 2

Abortion of reproductive organs in sweet pepper (*Capsicum annuum* L.): a review

Abstract

*Levels of abortion of reproductive organs (i.e. buds, flowers and young fruits) in sweet pepper plants (*Capsicum annuum* L.) are high, and cyclical fluctuations occur in fruit set. Stages susceptible to abortion are very young buds (< 2.5 mm), buds close to anthesis, and flowers and fruits up to 14 d after anthesis. An overview of factors and processes involved in flower and fruit abortion in sweet peppers is presented. More light, higher CO₂ concentrations, and lower planting density increase the availability of assimilates per plant, and decrease fruit abortion. The cyclical pattern in fruit set is caused by changes in demand for assimilates. High flower abortion occurs when fast growing fruits (at approx. 3 weeks after anthesis) are present, due to competition for assimilates. Fruit set increases when those fruits are almost mature and have a low assimilate demand. Prior to abortion, auxin export from the reproductive organ diminishes, ethylene production increases, and lower levels of activity of sucrose-cleaving enzymes are found. Severe water stress and low nutrient supply also increase abortion. Low night- and high day-time temperatures hamper pollen development, causing low seed set, which can result in fruit abortion. Two theories have been used to explain abortion: unbalanced demand for and supply of assimilates, and hormonal dominance of developing fruits over young fruits. Attempts to prevent abortion or diminish the cyclical pattern of fruit set have not yet been successful, but new suggestions are presented.*

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Introduction

Capsicum species, including sweet pepper, are grown in most countries, with an estimated total global production area of 3 million ha. Sweet peppers are cultivated in the field as well as in greenhouses. Some of the major production areas are China, Mexico, Spain, and the USA. (Bosland and Votava, 2000).

Abortion of reproductive organs is common in sweet pepper. Even when sweet peppers are grown in glasshouses, under carefully controlled production environments, abortion of reproductive organs occurs. Periods of high levels of abortion alternate with periods of low levels of abortion. Bakker (1989a) observed complete flower abortion during periods of high flower abortion, compared to 30% abortion during periods with low flower abortion. This cyclical pattern, or “flushing”, occurs more-or-less simultaneously for all growers in a particular region (Heuvelink *et al.*, 2004), resulting in several weeks of high yields and lower prices alternating with several weeks of low yields and higher prices. For the grower, this pattern of harvests makes planning for labour demand difficult (Heuvelink *et al.*, 2004).

Abortion is defined as the cessation of development and growth of an organ, after which it usually abscises. Reproductive organ that aborts can be buds, flowers, or young fruit. This paper refers to all these three stages, but the data cited do not always specify whether abortion concerned buds, flowers, or young fruit. We focus on abortion in sweet peppers, where approx. 70 – 80% of the reproductive organs abort and a cyclical pattern of fruit set occurs. Occasionally, we will refer to hot peppers.

Knowing which factors play a role in the abortion of reproductive organs, and what processes take place during abortion, could help to reduce the extent of abortion in sweet pepper. Here, we provide an overview of possible causes of flower and fruit abortion. First, factors which affect abortion are discussed, including environmental influences (e.g., light, CO₂, and/or temperature), the effects of plant growth and development (e.g., competition from other fruits), and management practices (e.g., pruning, or cultivar choice). Those physiological processes that take place during abortion in sweet pepper are then presented. Finally, attempts to prevent or diminish fruit abortion in sweet pepper are reported, with suggestions for future research.

Factors affecting abortion

Environmental factors

Light: Three aspects of light are important: intensity, duration (photoperiod) and quality (Ascough *et al.*, 2005). The effect of light intensity on fruit abortion has received most attention, whereas no reports have been found on the effects of light quality (i.e., different wavelengths) on fruit abortion.

Higher light intensities decrease abortion. Aloni *et al.* (1996) used light levels between 200 – 920 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in five cultivars of *Capsicum annuum* L., and recorded flower abortion after 15 d. Abortion in the bell-type cultivar ‘899’ decreased from 100% to 15% with increasing light intensity, while in the paprika cultivar ‘Lehava’, levels decreased from 21% to 0%. The other three bell-type cultivars showed intermediate levels of flower abortion. Turner and Wien (1994a) conducted a shading experiment (80% shading of incident radiation for 7 d.) using the cultivars ‘Ace’ and ‘Shamrock’. Bud and flower abortion levels for ‘Ace’, under unshaded conditions, were between 2% – 26%, and

between 7% – 43% under shaded conditions. For the cultivar ‘Shamrock’, abortion levels were 31 – 67% under unshaded and 54 – 85% under shaded conditions. Wien *et al.* (1989), Aloni *et al.* (1994), and Shifriss *et al.* (1994) also reported increased flower abortion when shading (40 - 80%) was applied.

Demers *et al.* (1998) extended the natural photoperiod December to July in Quebec, Canada, to 16, 20, or 24 hours with artificial light (high pressure sodium lamps; $110 \mu\text{mol m}^{-2} \text{s}^{-1}$). Photoperiods of 16, 20, and 24 h resulted in higher numbers of fruit per plant (equivalent to 78, 97, and 83 fruits m^{-2}) throughout the cultivation period, compared to the natural photoperiod (which produced 69 fruits m^{-2}), mainly due to higher yields in the Spring. The number of nodes was 10% higher in continuous light, resulting in more flowers, but equal fruit yields (in kg plant^{-1}). Fruit mass (g fruit^{-1}) was not significantly different between a normal photoperiod and the 20 h or 24 h photoperiod, implying that higher levels of fruit abortion occurred under (near) continuous light. In these experiments, however, the effect of photoperiod was confounded by light quantity, as these treatments with longer photoperiods also had a higher total quantity of light due to the use of artificial light sources (Demers *et al.*, 1998).

CO_2 : Nederhoff and Van Uffelen (1988) reported a level of fruit set of 55 fruits m^{-2} over 3.5 month period at a CO_2 concentration of $450 \mu\text{mol mol}^{-1}$, compared to 36 fruits m^{-2} at ambient levels ($344 \mu\text{mol mol}^{-1}$). Higher fruit yields under elevated CO_2 levels are reported by Aloni and Karni (2002), and by Dieleman *et al.* (2003), although levels of fruit abortion were not addressed. The former authors obtained 18 – 22% higher fruit yields (kg plant^{-1}) when CO_2 concentration was approx. $700 \mu\text{mol mol}^{-1}$ during the day compared to an ambient CO_2 concentration ($350 \mu\text{mol mol}^{-1}$). The latter authors obtained a 58% increase in the number of fruits harvested when the CO_2 concentration was increased from 380 to 780 $\mu\text{mol mol}^{-1}$. The effect of CO_2 on fruit abortion was apparent throughout the whole cultivation period, rather than in the short term, where fruit set also depended on the number of growing fruit (Dieleman *et al.*, 2007).

Temperature: Abortion levels in the bell-type cultivar ‘Delphin’, followed over a 19-week period, increased from 59% to 83% when the 24 h mean temperature was increased from 16°C to 24°C (Bakker, 1989a). Increasing daytime as well as night temperatures increased abortion, but the effect of night temperature was much clearer. Rylski and Spigelman (1982) reported that daytime temperatures of 22°C , 25°C , or 28°C (at a constant night temperature of 18°C) over a 3 month period did not have any effect on the level of flower abortion (61%) in the bell-type cultivar ‘Ma’or’. Decreasing the night temperature from 24°C to 15°C (at a day-time temperature of 26°C) decreased abortion from 88% to 70% in the same cultivar (Rylski and Spigelman, 1982). Aloni *et al.* (2001) reported only a slight increase in the extent of flower abortion under 8 d of heat stress ($32^\circ\text{C}/26^\circ\text{C}$ day/night) in the cultivar ‘Mazurka’ compared to $28^\circ\text{C}/22^\circ\text{C}$ (day/night), abortion levels being 9% and 6% abortion, respectively. In contrast, Marcelis *et al.* (2004) found that a constant temperature of 33°C for 4 days caused 100% abortion of buds and flowers in the same cultivar. A short-term exposure to high temperature (e.g., 6 h at 33°C) did not significantly influence the levels of abortion (30% – 55%) in the cultivars ‘Ace’ and ‘Bell Boy’; whereas, long-term exposure (> 48 h at 33°C) increased flower abortion to 61 – 92% (Erickson and Markhart, 2002). Aloni *et al.* (1991), Huberman *et al.* (1997), and Erickson and Markhart (2001) also reported increased abortion in buds, flowers, and young fruit caused by high temperatures ($> 30^\circ\text{C}$).

Relative air humidity: Baër and Smeets (1978) observed no differences in the percentages of fruit abortion (16 to 19% over a 1 month period) at constant relative humidities (RH) of 55, 80, or 95%, although the number of fruits set was lower at 95% RH. Fewer flowers were formed at 95% RH. The numbers of flowers and fruits were negatively correlated with RH during the night (Bakker, 1989b), while fruit abortion was negatively correlated with RH during the day (Bakker, 1989b). However, at high temperature (33°C) Erickson and Markhart (2001) found no effect of a constantly low RH (60%) on the numbers of flower buds and flowers over an 18 - 25 d period, compared to 75% RH. They therefore concluded that the high rate of fruit abortion at high temperatures was not due to water stress caused by a low RH.

Water and nutrient supply: Water stress (25% of normal evapotranspiration; ET) in the cultivar 'Blue Star' increased abortion to 83% compared to 51% at 100% ET (Jafaar *et al.*, 1994). However, González-Dugo *et al.* (2007) reported no difference in the percentage of fruit abortion (41-48%) under water stress of 75% ET compared to full water supply (100% ET) in the cultivar 'Sonora'. Total number of fruits in the cultivar 'Drago' was not affected when the water supply was 36-71% ET (Fernández *et al.*, 2005), although more fruits became deformed as water stress increased. Batal and Smittle (1981), Dalla Costa and Gianquinto (2002), and Antony and Singandhupe (2004) all reported higher fruit yields in sweet pepper when water stress was lowered, but no information on abortion levels was given. O'Sullivan (1979) mentioned that fruit abortion decreased when irrigation was applied at low rainfall, but did not give abortion levels. All experiments were performed in soil.

Maynard *et al.* (1962) applied three levels of nitrogen fertilisation (5.6, 11.1, and 22.2 mM N), 11 given three times a week, to the cultivar 'Pennwonder' growing in soil, and repeated the experiment twice. The level of nitrogen fertilisation had no effect on the total numbers of flowers. The lowest fruit abortion was 81% at 22.2 mM N in the first experiment and 68% at 11.1 mM N in the second experiment. Xu *et al.* (2001) applied three concentrations of nutrients to plants growing in perlite: low (3, 0.5, and 1.25 mM N, P and K), intermediate (twice this concentration), and high (three times this concentration). Fruit set percentage was comparable in all three treatments (44 – 48%) when all container volumes were small (9 or 18 l), but decreased from 43% to 36% when the nutrient concentration tripled in a large container (33 l). However, most flowers and fruits were produced when the highest concentration of nutrients was applied to large containers. Information on the influence of the level of nutrition on yields is more abundant (Batal and Smittle, 1981; Russo, 1991; Bar-Tal *et al.*, 2001). In general, fruit yield increased with increasing fertilisation levels.

The conductivity of the nutrient solution (from 2 - 10 dS m⁻¹) in the nutrient film technique had no effect on the number of fruits harvested per plant (Tadesse and Nichols, 2003), but the yield per plant decreased with increasing EC. However, Bakker (1989c) mentioned no effect of different EC levels on fruit production (kg m⁻²). Increasing the NO₃:NH₄ ratio, from 0.25 to 4, increased fruit yield per plant (Bar-Tal *et al.*, 2001).

Plant growth and development

Competition from previously formed fruits: Many researchers have found that the presence of previously formed fruits increases fruit abortion in different crops [e.g., in tomato (Bertin, 1995) and in melon (Valantin-Morison *et al.*, 2006)]. In the sweet pepper cultivar

'Mazurka', flower abortion was 100% when four fruit (each 10-15 d old) were present, compared to 88%, 58%, or 8% abortion when two, one, or zero fruit of this age were present, respectively (Marcelis *et al.*, 2004). The position(s) of the previously formed fruit also determined the level of fruit abortion. One fruit close-by (i.e., one-or-two nodes below) or distant (i.e., > eight nodes below) resulted in 0 -10% abortion, but a fruit three-to-five nodes below caused 30 - 50% abortion (Marcelis *et al.*, 2004). Fukumoto *et al.* (2004) reported a decrease in flower abortion when fruits were harvested sooner after flowering (e.g., 10 d after flowering; DAF) compared to harvesting 30 DAF.

Root growth: Plant growth decreased with a restricted rooting volume (NeSmith *et al.*, 1992; Xu *et al.*, 2001), which, in turn, decreased the fruit set percentage (Xu *et al.*, 2001). However, Karni *et al.* (2000) found no effect of root pruning (50% or 75% of roots removed) on the number of fruits or on fruit weight in bell-pepper.

Seeds: Higher seed numbers decreased the probability of abortion of the fruit itself, but increased the probability of abortion of fruits formed later (Marcelis and Baan Hofman-Eijer, 1997). The positive effect of seed numbers on the setting of a fruit was present when the number of seeds was low (<50), and maximum fruit set had already been reached at a relatively low seed number (50-100; Marcelis and Baan Hofman-Eijer, 1997).

Cultivation practices

Planting and shoot density: Planting density has an effect on the quantity of light which can be captured by each plant. Increasing the planting density from 1.6 to 4.6 plants m⁻² increased the level of abortion of flowers and young fruit per plant, from 69% to 86% (Marcelis *et al.*, 2004). Over a wide range of planting densities (1.3 – 53 plants m⁻²) in spice peppers, the number of fruit per plant decreased from 35 to 10 fruits plant⁻¹ (i.e., increased abortion) when the planting density increased from 1.3 to 10 plants m⁻², but the number of fruit per plant was more or less constant (\pm 5 fruits plant⁻¹) at higher planting densities (\geq 20 plants m⁻²; Caverro *et al.*, 2001). Planting density can also be increased by increasing the number of shoots (Cebula, 1995). A higher number of shoots per plant resulted in higher numbers of fruit per plant, but the increase was less than proportional, implying a higher percentage of flower and fruit abortion. Although lower planting density decreased flower abortion, this strategy is not used in practice, as the total yield ha⁻¹ decreases (Caverro *et al.*, 2001).

Pruning and fruit position: After each dichotomous branching, the larger branch is retained (main shoot), while the smaller branch is pruned after one or two leaves (side shoot). The intensity of pruning (i.e., how many nodes or leaves are left on the side shoot) influenced fruit abortion (Cebula and Kalisz, 2001). When more leaves and flowers are left on the side shoot, the number of fruit increased, but not as much as the number of flowers, implying a higher percentage of fruit abortion. Aloni *et al.* (1996) observed a higher flower and fruit abortion (40%) when there was a flower on the nearby side shoot, compared to 10% when no flower was present on this side shoot. The flower on the side shoot always aborted. Wubs *et al.* (2007) quantified, by survival analysis, the probability of abortion for flowers on the side shoot to be 21% higher than for flowers on the main shoot.

Cultivar: Fruit abortion percentages differed between cultivars if their fruit size differed (Wubs *et al.*, 2009a,b). Hot pepper cultivars with small fruits (2 – 4 g dry weight; DW) showed less fruit abortion (41 – 48 %) than sweet pepper cultivars with large fruits (9 – 14 g DW; 81 – 89 % abortion).

Table 2.1 Overview of cultivars of *Capsicum annuum* susceptible and less susceptible to flower and fruit abortion initiated by low light and high temperature stress

Type	Stress	Cultivar	Reference
Susceptible	Light	‘899’	Aloni <i>et al.</i> (1996)
		‘Maor’	Aloni <i>et al.</i> (1996)
		‘11480’	Aloni <i>et al.</i> (1996)
	Temperature	‘Shamrock’	Wien <i>et al.</i> (1989); Turner and Wien (1994a,b)
		‘Maor’	Aloni <i>et al.</i> (1994); Wien <i>et al.</i> (1993)
		‘Shamrock’	Turner and Wien (1994a)
Less susceptible	Light	‘Mazurka’	Aloni <i>et al.</i> (1996)
		‘Lehava’	Aloni <i>et al.</i> (1996)
		‘Lady Bell’	Wien <i>et al.</i> (1989)
		‘Ace’	Wien <i>et al.</i> (1989); Turner and Wien (1994a,b)
	Temperature	‘Lehava’	Aloni <i>et al.</i> (1994); Wien <i>et al.</i> (1993)
		‘Ace’	Turner and Wien (1994a); Erickson and Markhart (2001, 2002)
		‘Bellboy’	Erickson and Markhart (2001; 2002)

Furthermore, cultivars differ in their susceptibility to low light and high temperature stress (Table 2.1). It appeared that a cultivar which was susceptible to low light stress was often also susceptible to high temperature stress as well (Turner and Wien, 1994b). The periods of susceptibility to heat and low light stress were similar in the sweet pepper cultivar ‘Mazurka’ (Marcelis *et al.*, 2004). This could indicate that similar processes determine the susceptibility to heat and low light stress.

Period of susceptibility to abortion

A pepper flower or fruit is susceptible to abortion during only a part of its development. After surviving this critical period, a fruit will reach maturity. Most flowers of the sweet pepper cultivars ‘Gepetto’, ‘Nazar’, and ‘Funky’ aborted within 2 weeks after anthesis (Wubs *et al.*, 2009a). For hot pepper cultivars, the period of susceptibility was shorter (up to 10 d after anthesis). The period of susceptibility to flower abortion due to low light conditions in the pepper cultivar ‘Mazurka’ was from 1 week before anthesis until 2 weeks after anthesis (Marcelis *et al.*, 2004). The period of susceptibility to heat stress (33°C) in this cultivar was from shortly before anthesis until 2 weeks after anthesis, with a slight increase in abortion 12 d before anthesis (DBA; Marcelis *et al.*, 2004). The periods of susceptibility of buds to heat stress (33°C) in the cultivars ‘Ace’ and ‘Bellboy’ were 17 - 14 DBA and 5 - 1 DBA (bud length < 2.5mm and > 7.0 mm, respectively; Erickson and Markhart, 2002). Mature and fertilised flowers also showed increased abortion at 33°C (Erickson and Markhart, 2001; 2002), but Aloni *et al.* (1991) reported more abortion in buds than in flowers, and none in young fruits in the cultivar ‘Maor’ under heat stress.

Observations on fruit abortion in plant density experiments and cultivar experiments showed that only a small fraction (< 10%) of the flower buds abort before flower opening (Wubs, data not shown). The period of fruit set and early fruit growth is also susceptible to water stress (Dalla Costa and Gianquinto, 2002).

Processes taking place during abortion

Abortion in *Capsicum* is an active process. It takes place at the abscission zone, which is a morphologically distinguished layer of cells in the pedicel (Roberts *et al.*, 2000, 2002). When a fruit aborts, the middle lamina and cell walls in the abscission zone break down and the cells separate, after which, the fruit or flower abscises (Roberts *et al.*, 2000). Lieth *et al.* (1986) distinguished four stages of abscission in cotton bolls: stage 1, application of the stimulus; stage 2, beginning of the formation of the separation layer; stage 3, completion of the layer; and stage 4, abscission of the boll. The process can be stopped during the first two stages, but stage 3 and 4 are irreversible. Detailed information on the processes, at genetic and biochemical levels can be found in other reviews (Taylor and Whitelaw, 2001; Ascough *et al.*, 2005).

Plant growth, dry matter partitioning and enzyme activity

To unravel the processes occurring during abortion, stress conditions (applying 70-90% shading, or raising the temperature to 33°C - 35°C) were applied to cause abortion, and changes in plant growth and dry matter partitioning were measured. Light stress decreased net photosynthesis, total plant growth, relative growth rate (RGR), and net assimilation rate (NAR; Turner and Wien, 1994a,b; Aloni *et al.*, 1996). High temperature had a positive (Erickson and Markhart, 2001) or no effect (Aloni *et al.*, 1991) on these growth parameters. Organ respiration rate and sugar accumulation decreased in buds and flowers, a sign of decreased dry matter partitioning into the buds and flowers (Aloni *et al.*, 1991, 1997; Turner and Wien, 1994b). These decreases were higher in buds than in young leaves. Turner and Wien (1994b) and Aloni *et al.* (1996) concluded that altered dry matter partitioning was the primary factor causing high abortion under stress conditions, rather than a decrease in photosynthesis. When no light or heat stress is applied, but fast growing fruits are present, the shortage of assimilates due to competition from other fruit is likely to cause abortion.

The activity of sucrose handling enzymes, sucrose synthase and acid invertase, changed under stress. Acid invertase activity in buds decreased under heat stress (Aloni *et al.*, 1991), while sucrose synthase activity increased when light levels increased from 85 – 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Aloni *et al.*, 1997). Under temperature and light stress, reducing sugar concentrations were also lower (Aloni *et al.*, 1991; 1997). Aloni *et al.* (1997) suggested that high rates of sucrose supply support high metabolic activity, which ensured fruit growth and reduced fruit abortion.

Hormones

Under high temperatures, auxin concentration and auxin transport through the pedicel of flowers and fruitlets decreased (Wien *et al.*, 1993; Huberman *et al.*, 1997). At the same time, increased concentrations of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) were found, especially after longer periods of exposure (Wien *et al.*, 1989, 1993). This resulted in an increase in the production of ethylene in flowers and flower buds under high temperature stress (33°C) (Aloni *et al.*, 1994; Huberman *et al.*, 1997) or 80% shading (Wien *et al.*, 1989). High levels of ethylene increased the susceptibility to abortion. The sensitivity of the flower to ethylene, however, could also depend on auxin levels (Huberman *et al.*, 1997) or on sugar levels (Aloni *et al.*, 1997).

Limited information was found on the effect of other hormones on flower abortion in sweet pepper. In other crops, concentrations of abscisic acid (ABA) can be positively correlated with fruit abortion (Guinn and Brummett, 1987; Gomez-Cadenas *et al.*, 2000). The latter state that ABA mediates between assimilate supply and ACC. Guinn and Brummett (1988) state that an increased ratio of ABA over the auxin indoleacetic acid (IAA) precedes high rates of abortion. However, the correlation between ABA levels with assimilate uptake and abortion were contradictory (Ho *et al.*, 1983; Yang *et al.*, 2003). Gibberellin is needed for the fruit set of tomato (Serrani *et al.*, 2007) and inhibition of gibberellin synthesis increases fruitlet drop (Webster and Spencer, 2000).

Morphological and developmental processes

High temperature stress (33°C) during early bud development (i.e., bud length < 2.5 mm) resulted in pollen malformation and reduced pollen viability in the cultivars ‘Ace’ and ‘Bellboy’ (Erickson and Markhart, 2002). In the cultivar ‘Mazurka’, the number of pollen grains was largely unaffected by high temperature, but the germination ability was severely reduced (Aloni *et al.*, 2001). Decreasing night temperatures from 21°C to 10°C caused lower pollen viability and malformation of pollen (i.e., smaller grains, thinner exine) in the sweet pepper cultivar ‘Latino’ (Mercado *et al.*, 1997). The effect was only seen when small buds (< 5 mm) were treated. Pressman *et al.* (1998) reported decreased numbers of pollen grains and decreased ability to germinate, as well as a lower receptivity of the stigma, lower ability of the style to facilitate the growth of pollen tubes, larger ovaries, and shorter styles in the cultivar ‘Mazurka’ when night temperatures were decreased from 20°C to 10°C. The latter two effects were also reported by Polowick and Sawhney (1985) and by Shaked *et al.* (2004). When flowers grown at low or high temperatures were pollinated with pollen from flowers grown at intermediate temperatures, fruit shape and number of seeds were similar to fruits grown at intermediate temperatures (Polowick and Sawhney, 1985; Pressman *et al.*, 1998; Erickson and Markhart, 2002). Apparently, male floral organs were more affected by unfavourable temperatures than female organs. The affected stages were meiosis, tetrad formation, and start of the early microspore development (Mercado *et al.*, 1997; Erickson and Markhart, 2002).

Poor pollen development under unfavourable temperatures can be the cause of low seed numbers. Rylski and Spigelman (1982) reported a gradual decrease in the percentage of seedless fruits (from 34% to 1%) and an increase in seed numbers (52 – 106 seeds) when the night temperature increased from 15° to 24°C, at a constant daytime temperature of 26°C. An increase in daytime temperature from 22°C to 28°C (at a constant night temperature of 18°C) decreased seed number from 167 to 107. Aloni *et al.* (2001) found decreased seed number under high temperatures (32°C/26°C day/night). Seed set was also enhanced by higher RH during the day (Baër and Smeets, 1978), due to improved adhesion to the stigma and germination of pollen (Bakker, 1989b). Higher seed set increases the export of hormones from the fruit, thereby increasing the ability of the fruit to attract assimilates, and hence its sink strength.

Relation to cultivar differences

The differences in cultivar susceptibility to abortion (Table 2.1) are related to differences in the processes described above. The (relative) decrease in net carbon exchange rate, RGR, NAR, and respiration under light stress is higher in susceptible than in less susceptible cultivars (Turner and Wien, 1994a,b; Aloni *et al.*, 1996). Metabolic activity in, and dry

matter partitioning into, younger plant parts decreased in susceptible cultivars, while these parameters decreased in older plant parts in less susceptible cultivars, thereby increasing the chance of retaining the young leaves and flower buds (Turner and Wien, 1994b). Cultivar responses to heat or low light stress differ with respect to ethylene production (Aloni *et al.*, 1994; Wien *et al.*, 1989), their reaction to exogenous applied ethylene (Aloni *et al.*, 1994) and auxin export (Wien *et al.*, 1993). The effect of low night temperatures on flower and fruit development also differed between cultivars (Pressman *et al.*, 1998; Shaked *et al.*, 2004).

Theories about the cause of flower and fruit abortion

There are two theories on possible causes of flower and fruit abortion in peppers. One concerns the competition between fruits for assimilates (Marcelis *et al.*, 2004), while the other is related to hormone flows generated by the fruits (Bangerth, 1989, 2000). It has also been suggested that both theories are complementary. Marcelis *et al.* (2004) and Aloni *et al.* (1997) state that the availability of assimilates is the triggering factor for hormone production. Part of the experimental results found by Marcelis *et al.* (2004) and Marcelis and Baan Hofman-Eijer (1997) can only be explained by a combination of competition and dominance.

Source and sink strength

In the first theory (competition), the terms source and sink strength are used. These refer to the supply of, and demand for, assimilates, respectively. An increase in source strength increases fruit set, and an increase in sink strength decreases fruit set (Marcelis *et al.*, 2004).

Source strength comprises all those factors that influence the availability of assimilates, such as light levels, CO₂ concentration, and planting density. Temperature also influences source strength, as the photosynthesis rate at high radiation levels (2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) was higher under high (27°C) than under low (17°C) temperatures (Dieleman *et al.*, 2003). When the temperature is higher than 30°C, the rate of photosynthesis decreased (Niu *et al.*, 2006).

Total plant sink strength consists of generative and vegetative components. The generative sink strength is the total of the sink strengths of all the individual fruit, which depends on the developmental stage of the fruit (Marcelis and Baan Hofman-Eijer, 1995), cultivar (Fig. 2.1, Wubs *et al.*, 2009b) and the number of seeds per fruit (Marcelis and Baan Hofman-Eijer, 1997). The vegetative part of a plant is also a sink, as it needs assimilates for growth of the stem and leaves (Aloni *et al.*, 1991, 1996). Temperature could also affect the sink strengths of the fruits and the vegetative parts. This is investigated in Chapter 3 and Chapter 6.

Together, source and sink strength can explain the cyclical fluctuations in fruit set. An increase in source strength increases fruit set, resulting in more fruits and therefore an increased sink strength. An increase in sink strength decreases fruit set, which, after a while, results in a lower sink strength, which in turn allows for an increased fruit set.

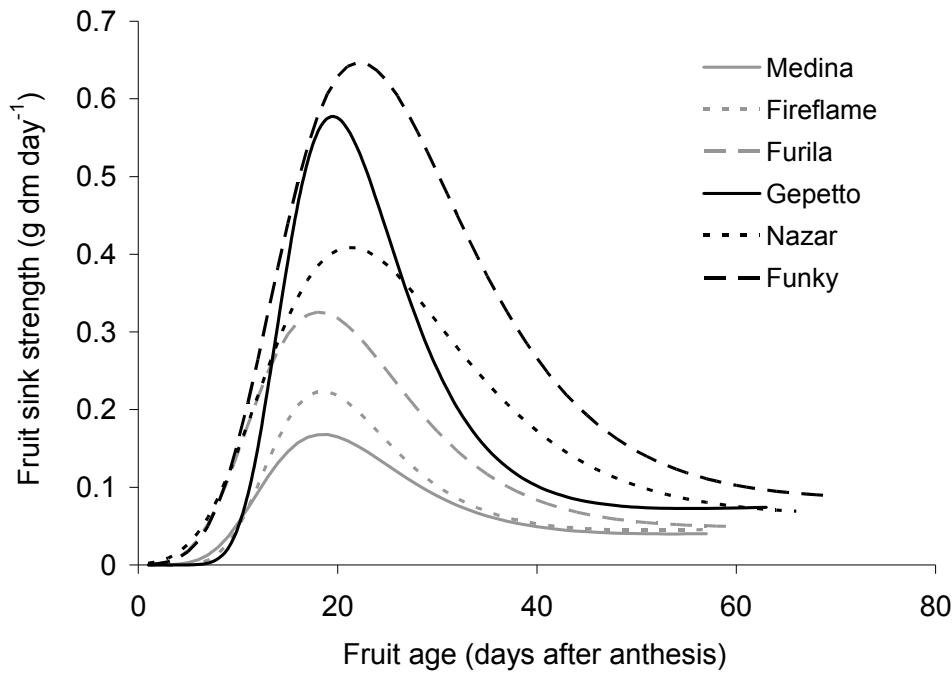


Fig. 2.1 Fruit sink strength of different cultivars of *Capsicum annuum* L., where sink strength is defined as the potential growth rate.

Hormonal dominance

The theory involving hormonal dominance of one fruit over the other was proposed by Bangerth (1989, 2000) and is called “correlatively-driven abscission”. It is based partly on the general model of organ abortion where the balance between auxin and ethylene determines whether or not an organ will abort (Taylor and Whitelaw, 2001). This model was also assumed to be valid for fruit abortion in sweet pepper (Wien *et al.*, 1993; Huberman *et al.*, 1997). Auxin is produced by the leaves or fruit and is translocated through the petiole, basipetally. As long as the flux of auxin is maintained, it prevents the formation of an abscission layer at the base of the organ. A decline in auxin export is an early sign of abscission. When an organ begins to senesce, it produces ethylene which inhibits the production and export of auxin, increases the sensitivity of the cells in the abscission layer to ethylene, and an abscission layer can be formed (Taylor and Whitelaw, 2001). In fruits, seeds are the main producers and exporters of auxins, but the growth points of the vegetative organs also export auxins. In the “correlatively-driven abscission” theory, auxin export from older fruits inhibits auxin export of younger fruit, thereby promoting the formation of an abscission layer in the younger fruit (Bangerth, 1989, 2000). Temperature and humidity can influence the number of seeds, and hence the hormone flows, thereby influencing abortion.

With respect to the alternation of groups of nodes with fruit, and groups of consecutive nodes without fruit, this would mean that the flow of hormones varied with the developmental stage of the fruit, or that its influence diminished when the distance between the fruits was greater.

Synthesis

A schematic overview of those factors that can affect abortion of a particular fruit is given in Fig. 2.2. The diagram is presented based on the source and sink strength theory, as many factors can be easily explained by this theory. Some factors, however, can not be explained by this theory. The position of a flower, whether it is situated on the main or side shoot, influences its probability of abortion, but it is unlikely that this can be explained by source or sink strength. Hormonal dominance of a fruit located on the main branch might be more likely, but architectural effects (e.g., development of xylem vessels) are also a possibility (Diggle, 1995). Other factors can be explained by both theories. A higher probability of fruit set, with increased seed set, may be caused by a greater sink strength of the fruit, but could also be due to increased hormone production and export.

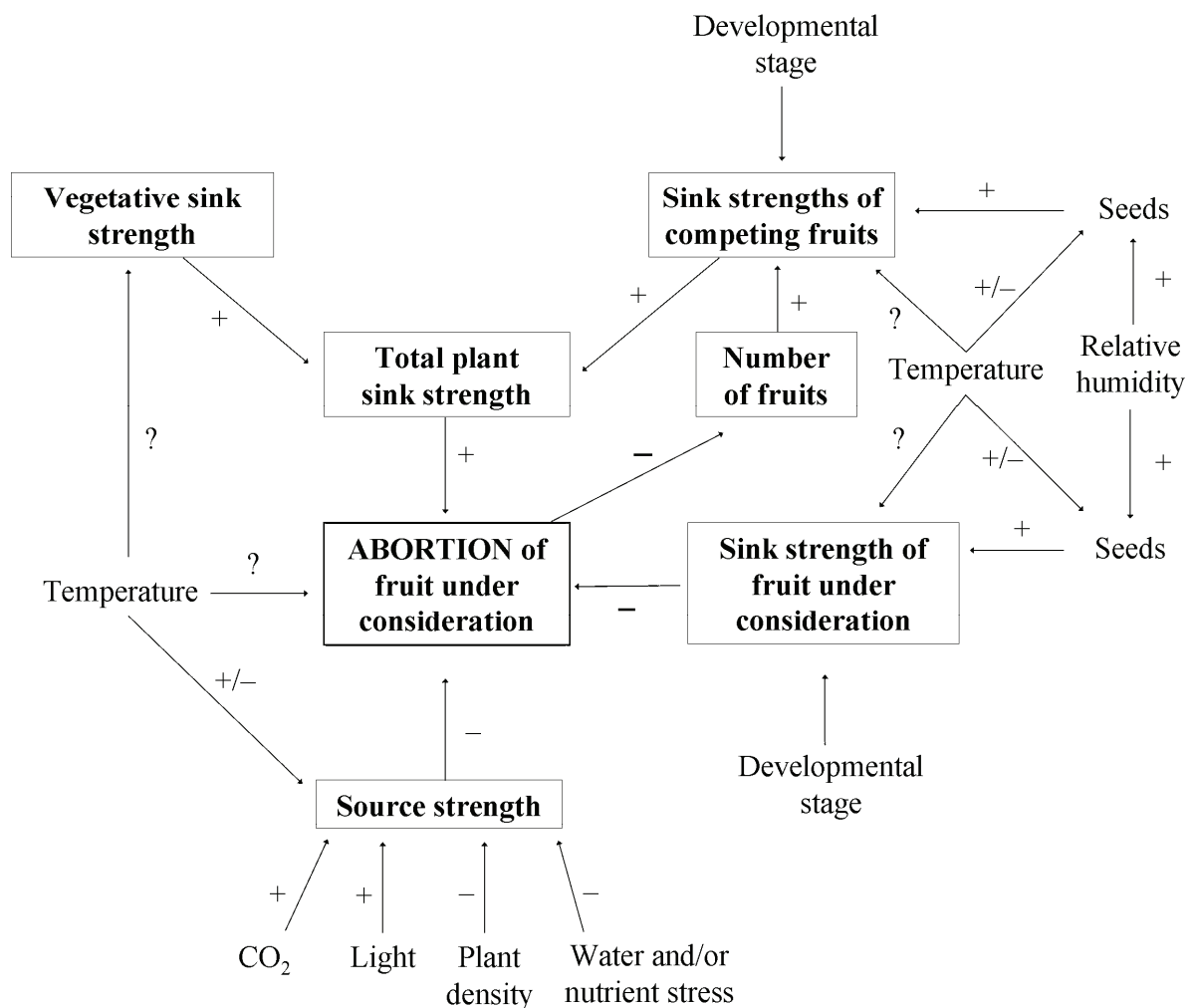


Fig. 2.2 Factors affecting fruit abortion in sweet pepper (*Capsicum annuum* L.). A positive sign implies that the factor increases fruit abortion, a negative sign means that it decreases flower and fruit abortion. +/- indicates that there is an optimum in the influence of the factor, and ? means that the effect has not yet been established.

Attempts to realise more regular fruit set and lower fruit abortion

Attempts have been made to minimise the level of abortion of fruit and/or diminish the cyclical pattern of fruit set in sweet pepper. Heuvelink *et al.* (2004) and Buwalda *et al.* (2009) tried to create two compartments with antagonistic patterns of fruit set. The former workers applied two planting dates (4 weeks apart). This only resulted in more continuous fruit set (i.e., the total of the two compartments) when the crop was planted later-than-normal (i.e., April and May). Normal planting dates (December and January) resulted in antagonistic fruit set in the first flush, but fruit set in the two compartments was synchronised during the rest of the cultivation period. Buwalda *et al.* (2009) tried to establish a phase-shift in fruit set in one compartment by temperature regulation, while, in the other compartment, no treatment was given (normal fruit set pattern). The combined fruit set of both compartments was more regular, but the phase-shift was difficult to establish when the outside temperature was high. Fruit pruning, aimed at two set fruit per plant per week, did not result in a completely regular harvest pattern (Heuvelink *et al.*, 2004). Artificially creating parthenocarpic fruit (i.e., fruit without seeds), by applying auxin to the stamen, greatly decreased the fluctuations in the pattern of fruit set (Heuvelink and Körner, 2001). However, as there are no commercial, parthenocarpic varieties of pepper, and creating parthenocarpic fruit by applying chemicals is troublesome, it is difficult to use this method in commercial practice. Wien *et al.* (1993) and Wien and Zhang (1991) applied an ethylene inhibitor (silver thiosulphate) to the crop, which reduced abortion, but it resulted in deformed fruit. Moreover, the toxicity to humans of the chemicals applied limits this strategy in commercial practice (Wien *et al.*, 1993). Although *Capsicums* species are essentially self-fertilising crops, using bees to assist pollination might improve fruit set, especially under otherwise less favourable circumstances. Extra pollination increases seed set (Pressman *et al.*, 1989; Marcelis and Baan Hofman-Eijer, 1997), and, at low seed set, more seeds imply higher probability of fruit set. However, experiments in which pollination with bees was applied did not result in higher fruit set compared to self-pollinating flowers (De Ruijter *et al.*, 1991; Cruz *et al.*, 2005).

Conclusions and areas for further research

In general, when sweet peppers are produced in a controlled environment, at higher latitudes, the main factors causing abortion will be light limitation during the Winter season, high temperatures in the Summer, and competition between fruits during the whole growth season. When sweet pepper is grown under field conditions at lower latitudes, light is a less limiting factor, but high temperature stress and water limitation are the main causes of abortion. Management of nutrients is also more difficult in field conditions. Cultivar choice can also be an important factor, especially under stress conditions. Processes which occur during abortion (e.g., alternative assimilate partitioning, decrease in sugar content, and lower enzymatic activities) are more or less similar during low light and heat stress (Turner and Wien, 1994b). Attempts to alleviate the fluctuations in fruit set have not been very successful so far. Parthenocarpic fruits are promising, but are not yet commercially available.

As regards biochemical and physiological processes during abortion, there are fundamental questions still to be answered. There have not yet been any investigations in relation to the four phases of fruit abortion stated previously. How long do the abortion processes take, and when does the process become irreversible? How do hormone flows (especially auxins) change with the stage of fruit development?

Further possibilities to increase fruit set, or to diminish fluctuations in fruit set, could also be investigated. Dieleman *et al.* (2007) suggest that fluctuations in fruit set might be regulated by controlling the supply of CO₂. Lower levels of CO₂ should be given during periods of fruit set, in order to avoid high fruit set followed by periods of high fruit abortion some weeks later, and CO₂ supply should be increased during period of low fruit set.

Management options from other crops might be worth trying. In apple, bending of the branches was used to slow down vegetative growth, which promoted early flower formation (Tromp, 1968). Flower production was also higher (Tromp, 1970). Robbie *et al.* (1993) reported higher final numbers of set fruit when shoots of apple trees were growing in a horizontal position rather than in a vertical position. Preliminary experiments in sweet pepper plants with bent stems have shown that vegetative growth decreased and generative growth increased, but that total plant growth and the numbers of set fruit were lower in bent shoots (Marcelis and Heuvelink, unpublished data). Heating of the roots could be an other option. Research in this field has been mainly done on tomato, and the results are rather equivocal. Whether or not the effect on yield was beneficial varied with season (Gent and Ma, 1998) and air temperature (Gosselin and Trudel, 1983; Papadopoulos and Tiessen, 1983). Delays in fruit ripening in plants with heated roots were reported by Orchard (1980), but not by Moss (1983) and Papadopoulos and Tiessen (1983). During the final weeks of cultivation, the apical growth points can be removed as growth of the vegetative part is no longer needed and all assimilates can be invested in the fruit. When the top of a plant is removed (three nodes above a flowering node) fruit abortion in the six flowers below the point of pruning was 33%, compared to 58% in control (unpruned) plants (Marcelis and Heuvelink, unpublished data).

The use of different wavelengths might also be worth exploring. Abscission of leaf explants of *Coleus* was prevented by red light (Craker *et al.*, 1987) and flower abscission in Hibiscus was delayed when the leaves were exposed to red light (Van Meeteren and Van Gelder, 2000). Far-red light enhanced or accelerated abscission. The increasing use of light-emitting diodes (LEDs; Hogewoning *et al.*, 2007) could facilitate this option, as LEDs emit light of a relatively narrow spectrum.

Chapter 3

Quantifying growth of sweet pepper fruits non-destructively

Abstract

The goal of this chapter is to describe accurately fruit dry weight growth of sweet peppers using simple mathematical functions. Fruit growth of sweet pepper was measured non-destructively in an experiment with three different average daily temperatures (18°C, 21°C and 24°C) and in an experiment with six cultivars with different fruit sizes. Measurements of fruit length and fruit diameter or circumference were performed twice a week. On the basis of these measurements fruit volume was estimated. A linear relationship related fruit fresh weight to estimated fruit volume, and a Ricker or polynomial function related fruit dry matter content to fruit age. These relationships were used to convert estimated fruit volume into fruit fresh and dry weight. Four sigmoid functions were used to quantify the growth of sweet pepper fruits: Logistic, Gompertz, beta growth, and Richards function. Fitting a sigmoid function through the dry weight data was not possible, as the dry weight growth was clearly non-zero at harvest and does therefore not reach a plateau as in the functions used. The growth functions were therefore fitted to the fresh weight growth of the fruits. The Richards function was the best function in each data set, closely followed by the Gompertz function. Temperature hardly affected fruit growth, whilst the cultivar influenced the asymptote of the functions as well as the parameter k describing the curvature of the function. The fruit dry weight growth is obtained by multiplication of the sigmoid function and a polynomial or Ricker function relating fruit dry matter content to fruit age.

Introduction

In many crops, the size and weight of an organ (e.g. fruit, leaf head of cabbages) are important parameters determining the price of the product. By following the growth of an organ, final fruit size can be estimated. These measurements can be taken in two ways. The first method is to regularly harvest fruits (Nielsen *et al.*, 1991; Hubbard and Pharr, 1992; Arena and Curvetto, 2008). Another method of following fruit growth over time is by performing repeated non-destructive measurements of the dimensions of fruits. Examples are length and diameter measurements of chili peppers by Pagamas and Nawata (2008), length of cacao fruits (Daymond and Hadley, 2008) and diameter of highbush blueberry fruits (Godoy *et al.*, 2008), loquat (Cuevas *et al.*, 2003), pear (Garriz *et al.*, 2005) and apple (Al-Hanai and Roper, 2004; Greer, 2005). Repeated non-destructive measurements of the same fruit are statistically speaking preferred as the variance between measurements of the same fruit is smaller than for measurements of different fruits (De Silva *et al.*, 1997). However, repeatedly touching the fruits for measurements might affect their growth negatively.

In contrast to fruit dimensions, fruit weight cannot be measured directly in repeated non-destructive measurements. Intermediate functions should then be used to convert the fruit dimensions into fresh and/or dry weight. Marcelis (1992b, cucumber) and Marcelis and Baan-Hofman Eijer (1995, sweet pepper) used a linear relationship to relate fresh weight to fruit volume (the volume was obtained from non-destructive measurements). A polynomial function between fraction dry matter of the fruit and fruit age was used to convert fruit fresh weight into fruit dry weight. Cuevas *et al.* (2003) followed fresh weight growth of loquat over time by relating fresh weight to equatorial diameter using a linear relationship. In apple, more complex functions were needed to relate fruit dimensions to fruit weight (De Silva *et al.*, 1997).

For predictions of individual fruit weight and for modelling purposes quantification of fruit growth over time is needed. Growth of organs often follows a sigmoid growth curve which can be described using functions such as the Logistic, Gompertz and Richards function. These functions have been used to describe fruit dimension (Adams *et al.*, 2001; Cuevas *et al.*, 2003; Garriz *et al.*, 2005; Barrera *et al.*, 2008; Daymond and Hadley, 2008), fruit fresh weight (Marcelis, 1992b; Tadesse *et al.*, 2002; Barrera *et al.*, 2008) and fruit dry weight (Marcelis, 1992b; Barrera *et al.*, 2008) over time. Sigmoid functions are also often used for describing other biological processes such as whole crop growth or seed germination. In these cases, comparison of sigmoid functions on the same data set have been done (Brown and Mayer, 1988; Yin *et al.*, 2003). Comparison of different sigmoid functions has hardly been performed for growth of individual fruits.

This Chapter aims at comparing simple growth functions fitted to data regarding growth of individual sweet pepper fruits, the dimensions of which were measured non-destructively. We test which of the most commonly used functions, namely the Logistic, Gompertz and Richards function, are most suitable to describe fruit growth in *Capsicum*. We also applied the beta growth function. This function was introduced by Yin *et al.* (2003), but has not yet been used for fruit growth. As the purpose is to describe fruit growth expressed in weight, attention is also paid to the intermediate functions by means of which the fruit dimensions can be related to fruit weight. The question is answered whether or not it is more appropriate to fit the sigmoid function on fresh fruit weight or dry fruit weight.

Two experiments were used, one in which one cultivar was grown at three different average daily temperatures and one with six different cultivars with varying fruit sizes.

Materials and Methods

The method used to derive fruit dry weight growth is similar to the one used by Marcelis and Baan Hofman-Eijer (1995). Non-destructive measurements of fruit length and diameter over time were performed to estimate fruit volume. Fruit volume is converted into fruit fresh weight using an intermediate function relating fruit fresh weight to estimated fruit volume. Fruit fresh weight is converted into fruit dry weight using a second intermediate function relating fraction dry matter of the fruit to fruit age. The age of the fruit non-destructively measured was known at each measurement.

Sigmoid functions

The Logistic, Gompertz, and Richards functions are often used to describe fruit growth. These functions are all able to describe fruit growth in time with one mathematical formula. The parameters of the functions can be interpreted easily. The Richards function, or general logistic function (equation 3.1), is the most generic function. It has four parameters, by means of which it is able to describe asymmetric fruit growth curves, e.g. growth curves which are not point-symmetric around the inflection point.

$$w(t) = \frac{w_{\max}}{(1 + v e^{-k(t-t_m)})^{1/v}} \quad \text{equation 3.1}$$

$w(t)$ is the weight at time t (time after anthesis), w_{\max} is the upper asymptote, k a constant determining the curvature of the growth pattern, t_m the position of the inflection point where the growth rate is maximum and v is a shape parameter. The parameter v determines whether the upper or lower asymptote is approached more gradually: for $v < 1$ the lower asymptote is approached more gradually than the upper asymptote, while the opposite holds for values of $v > 1$.

If the shape parameter v has the value of one, the Richards function reduces to the Logistic function (equation 3.2).

$$w(t) = \frac{w_{\max}}{1 + e^{-k(t-t_m)}} \quad \text{equation 3.2}$$

Parameters have similar interpretations as in the Richards curve. The sigmoid curve described by the Logistic function is point-symmetric around t_m .

When the value of v approaches zero, the Richards function approaches the Gompertz function (equation 3.3). Although the Gompertz function has one parameter less than the Richards function, it is also able to describe an asymmetric sigmoid curve. However, it is less flexible, as the lower asymptote is always approached more gradually than the upper asymptote.

$$w(t) = w_{\max} e^{-k(t-t_m)} \quad \text{equation 3.3}$$

Yin *et al.* (2003) introduced the beta growth function (equation 3.4) with three easy interpretable parameters, which was able to describe growth of several crops and plants.

$$w(t) = w_{\max} \left(1 + \frac{t_e - t}{t_e - t_m}\right) \left(\frac{t}{t_e}\right)^{\frac{t_e}{t_e - t_m}} \quad \text{equation 3.4}$$

Parameter t_e indicates the end of the growth period. w_{\max} and t_m have similar interpretations as in the other functions. Weight at $t = 0$ is exactly zero and the function has exactly the value w_{\max} at time t_e . This is in contrast to other functions, where zero and w_{\max} are asymptotes of the function. A constraint should be set that $w_t = w_{\max}$ when $t > t_e$, in order to get an upper asymptote. If this is not done, the function decreases after the point (t_e, w_{\max}) .

Intermediate functions

Two intermediate functions are needed to eventually attain fruit dry weight growth over time from non-destructive measurements. The first is the relationship between fruit fresh weight and estimated fruit volume. For pepper fruits, a linear relationship seems appropriate (Marcelis and Baan Hofman-Eijer, 1995).

The second relationship describes fruit dry matter content as a function of fruit age. Dry matter fraction of a pepper fruit (g dw/ g fw) initially decreases rapidly after anthesis, but increases towards maturity. Marcelis (1992b) and Marcelis and Baan Hofman-Eijer (1995) used a third-order polynomial function for cucumber and a fourth-order for sweet pepper respectively to describe the relationship between fruit dry matter content (fdm) and fruit age (equation 3.5). The part between brackets is only included in the fourth-order polynomial.

$$\text{fdm}(t) = a_p + b_p t + c_p t^2 + d_p t^3 (+e_p t^4) \quad \text{equation 3.5}$$

Where t is the time after anthesis and a_p , b_p , c_p , d_p and e_p are parameters. The parameters in this function have no biological interpretation. Two other functions capable of describing the form of this relationship are the sum of two exponential functions (equation 3.6) and a modified Ricker function (Bolker, 2008) (equation 3.7).

$$\text{fdm}(t) = a_s e^{(b_s t)} + c_s e^{(d_s t)} \quad \text{equation 3.6}$$

$$\text{fdm}(t) = c_R - a_R (t - d_R) e^{(-b_R(t-d_R))} \quad \text{equation 3.7}$$

In the sum of two exponentials, a_s and c_s determine the value at $t = 0$ and b_s and d_s are the relative growth rates. The Ricker function has an asymptote, c_R . Parameter b_R and d_R determine the position of the minimum.

Experiments

Fruit growth of pepper was measured non-destructively in two experiments. The first experiment looked at fruits of one cultivar growing at three temperatures, whilst in the second experiment, fruit growth was measured on six different cultivars. The number of fruits measured non-destructively in both experiments is given in Table 3.1.

Temperature experiment: The experiment was conducted in three air-conditioned compartments (2.5 m by 5.4 m) of a Venlo-type glasshouse with *Capsicum annuum* cv. ‘Mazurka’ (RijkZwaan, De Lier, the Netherlands). The average temperatures were set at 18°C, 21°C and 24°C, with a difference of four degrees between day and night. Daytime was between 8am and 6pm, and night between 8pm and 6am, and the hours in-between were used for heating up and cooling down respectively. The average temperatures actually achieved were close to the set points: 18.3°C ± 0.5, 20.3°C ± 0.35 and 23.3°C ± 0.31 (mean ± sd). Vapour pressure deficit was kept constant at 0.7 kPa, implying an average relative humidity of 67%, 73% and 77%, for 18°C, 21°C and 24°C respectively. Plants were grown in 15 l pots, filled with commercial potting medium, at a density of 4.7 plants m⁻². Water was given daily, while fertilization with a standard nutrient solution (PG-mix, 12+14+24+2) was given once or twice a week depending on the plant growth. The experiment was performed between March and July. The average daily global outside radiation was 15.5 ± 2.9 MJ m⁻² d⁻¹ (mean ± sd).

Cultivar experiment: An experiment with six *Capsicum annuum* cultivars with different fruit fresh weights (between brackets as given by the seed company De Ruiter Seeds, the Netherlands) was conducted. ‘Medina’ (20 g), ‘Fireflame’ (20 g) and ‘Furila’ (45 g) were hot pepper cultivars, ‘Gepetto’ (135 g) was a cultivar with pointed sweet peppers and ‘Nazar’ (140 g) and ‘Funky’ (205 g) produced block-type sweet peppers. Plants were grown in a Venlo-type glasshouse compartment (12 m by 12.8 m) on rockwool substrate in Wageningen, the Netherlands, in a complete randomised block design, between April and September at a density of 3.8 plants m⁻². Average temperature was 21.6 ± 2.0°C (mean ± sd) and average daily global outside radiation 16.3 ± 5.6 MJ m⁻² d⁻¹.

Measurements and intermediate calculations

For non-destructive measurements of fruit growth, one or two flowers per plant were tagged at anthesis, and their anthesis date was recorded. Other fruits and flowers from these plants were removed, and newly developed flowers were removed weekly. Twice a week, length and diameter (cultivar experiment except for ‘Gepetto’) or circumference (temperature experiment, and cultivar ‘Gepetto’ in the cultivar experiment) of the tagged fruits were measured using a calliper or tape measure. Diameter and circumference were measured in the middle of the fruit. Fruits were harvested when completely red, and fresh and dry weight were measured at this time. In addition, fruits of various sizes were harvested from other plants and their fresh weight, length and diameter or circumference were measured to establish the relationship between fruit fresh weight and estimated fruit volume. Another group of flowers was also tagged at anthesis and harvested at different ages to obtain a relationship between dry matter fraction of the fruit and fruit age. The dry weight of fruits was obtained by drying the fruits in the oven for two nights at 105°C.

Fruit volume V was calculated from length l and diameter d as $V = \frac{1}{4} \pi l d^2$ or from length l and circumference c as $V = \frac{1}{4\pi} l c^2$, assuming a cylindrical fruit shape.

For the temperature experiment, the ages of the fruits were converted into degree-days, assuming a base temperature of 10°C (Marcelis *et al.*, 2006). The ages of the fruits in the cultivar experiment were scaled to the actual fruit growth duration when harvested when mature, or to the average fruit growth duration (Table 3.1, defined as 1) when harvested before maturity. Anthesis was set at zero.

After fruit volume was calculated for the fruits repeatedly measured non-destructively, the estimated fresh weight at each measurement was calculated using the relationship between fruit fresh weight and estimated fruit volume. Subsequently, fruit fresh weight was converted into fruit dry weight using the function relating fruit dry matter content to fruit age. The age of the fruits was known at each measurement. Underestimations or overestimations in the final calculated dry weight were corrected to the measured dry weight by the ratio (measured dw /calculated dw). This ratio was applied to the full growth curve of the particular fruit (i.e. at all measurement times).

Statistical set-up and analysis of curve fitting

The relationship between fruit fresh weight and fruit volume was fitted per experiment taking into account the treatment in the experiments (temperature and cultivar). No intercept was fitted. It was tested whether or not different slopes were needed for the treatment levels (temperature and cultivar).

Fruit dry matter content was arcsine-transformed before analysis to normalise the data (Sokal and Rolf, 1994). For purposes of simplicity the fruit dry matter content in equation 3.5-3.7 is given as the dependent variable. Equation 3.5 was fitted with linear regression analysis, while non-linear regression analysis was used to fit equations 3.6 and 3.7. The data of the temperature experiment were complemented with the data on fruit dry matter content and fruit age from Marcelis and Baan Hofman-Eijer (1995), which were conducted at 20°C. For the temperature experiment, no distinction was made between the temperatures and the two experiments. The number of data points of young fruits (less than 20 days) in the cultivar experiment was small for some cultivars. Parameters with a large

Table 3.1 Number (N) of fruits measured non-destructively and average fruit growth duration in the temperature and cultivar experiments.

Experiment	Treatment	N	Fruit growth duration (d)
Temperature	18°C	17	88
	21°C	23	76
	24°C	6	64
Cultivar	‘Medina’	74	55
	‘Fireflame’	70	57
	‘Furila’	51	59
	‘Gepetto’	29	66
	‘Nazar’	53	66
	‘Funky’	33	69

influence on the first part of each function (parameters a_s and b_s in the bi-exponential function, parameters b_R and d_R in the Ricker function and the intercept and first order term in the polynomial) were therefore assumed to be the same for all cultivars. The non-destructive measurements of fruit growth took place on individual fruits resulting in numerous individual growth curves. This made the fitting process different from destructive measurements where one average curve is obtained. Averaging all measured growth curves to one growth curve per treatment would have resulted in loss of information, just as averaging the parameters of individual fits on each fruit would do. Fitting the functions to the fruit weights in time was therefore performed using non-linear mixed models. This method takes into account that all measurements of one fruit are dependent on each other and that less variation exists between the measurements within one fruit than between measurements of different fruits. The method estimates the average values and standard deviations for function parameters (w_{max} , k , t_m etc). The growth of a specific fruit is described by a set of parameters sampled from these normal distributions. The deviation of a parameter of a specific fruit from the mean parameter value is the random effect. The mean value of each parameter can depend on treatment levels, in this case temperature and cultivar. Parameter ν in the Richards function was fitted without random effect because convergence failed when this was tried. The initial parameter estimates for the Richards function were the parameters of the Logistic curve, with the initial estimate for parameter ν set to one.

First, a fit was made with common parameters for all levels of factor. Convergence failed for all models when all parameters were dependent on treatment level. Forward selection was therefore done by making the parameters dependent on treatment level one by one and selecting the best model. In the best model, the other parameters were subsequently made dependent on factor levels as well, until the best model was obtained. For the best model, a first-order autocorrelation between the measurement points was inserted and it was checked whether the random effect was needed for each parameter.

To test whether two nested models, i.e. one model is a reduced form of another model, were different, a log-likelihood ratio test was performed. For the sigmoid growth functions, the goodness of fit is expressed as the log-likelihood and the Akaike's Information Criterion (AIC, equation 3.8), which takes into account the log-likelihood of the model as well as the number of parameters. Furthermore, the R^2 of the regression of the fitted values (y) against the observed values (x), as well as the mean deviation (MD, equation 3.9) and the root mean square error (RMSE, equation 3.10) between fitted and observed values.

$$AIC = -2 * \log \text{likelihood} + 2 * p \quad \text{equation 3.8}$$

$$MD = \frac{1}{n} \sum (P_i - O_i) \quad \text{equation 3.9}$$

$$RMSE = \sqrt{\frac{1}{n} \sum (P_i - O_i)^2} \quad \text{equation 3.10}$$

p is the number of parameters in the model, n is the number of data points, P_i is the predicted value of observation i and O_i is the observed value of observation i . For the best model inspection of the residuals was done (normality and independence of time).

All fits were done in R 2.6.0 (R core development team, 2008). The nlme package was used for fitting the mixed models (Pinheiro *et al.*, 2008).

Results

Relationship between fruit fresh weight and estimated fruit volume

In both experiments, the relationship between fruit fresh weight and estimated fruit volume was fitted well with a linear regression without intercept ($R^2 = 0.99$ for both fits). At 24°C, the slope was lower than at 18°C and 21°C, implying a lower fruit fresh weight at the same fruit volume (Table 3.2, $P < 0.001$, residual st. error 10.8). In the cultivar experiment, a model with different slopes fitted better to the data than one with a common slope (Table 3.3, $P < 0.001$, residual std. error 8.5). The slopes for ‘Medina’ and ‘Fireflame’ (hot peppers with similar shape and weight) were not significantly different ($P = 0.36$), but fitting a common slope for ‘Nazar’ and ‘Funky’ (sweet peppers with similar shape) gave a worse fit ($P = 0.001$).

Relationship between fruit dry matter content and fruit age

All three functions, the fourth–order polynomial, the bi-exponential function and the Ricker function, fitted equally well in the temperature experiment (Table 3.3, Fig. 3.1). The fourth– order polynomial function fitted best in the cultivar experiment (Table 3.3, Fig. 3.2). Fruit dry matter content was influenced by the cultivar in the second part of the growth (after 30 days). Fitting one function for either ‘Medina’ and ‘Fireflame’, or for ‘Gepetto’, ‘Nazar’ and ‘Funky’ resulted in a worse fit for all functions ($P < 0.001$). In both data sets, the fruit dry matter content seemed to level off in the oldest measured fruits, but this was beyond the age of the average time of harvest (Table 3.1, 780°Cd for the temperature experiment).

Table 3.2 Slope of relationship between fruit fresh weight and estimated fruit volume in the temperature and cultivar experiment. Slope is given in g fresh weight cm⁻³, N represents the number of data points in each regression. Values between brackets are the standard errors of the slopes. R^2 was 0.99 for both experiments.

Experiment	Treatment	N	slope
Temperature	18°C	196	0.49 (0.003)
	21°C	198	0.49 (0.003)
	24°C	139	0.41 (0.003)
Cultivar	‘Medina’	238	0.59 (0.015)
	‘Fireflame’	186	0.62 (0.020)
	‘Furila’	178	0.52 (0.008)
	‘Gepetto’	200	0.35 (0.002)
	‘Nazar’	342	0.43 (0.002)
	‘Funky’	288	0.43 (0.002)

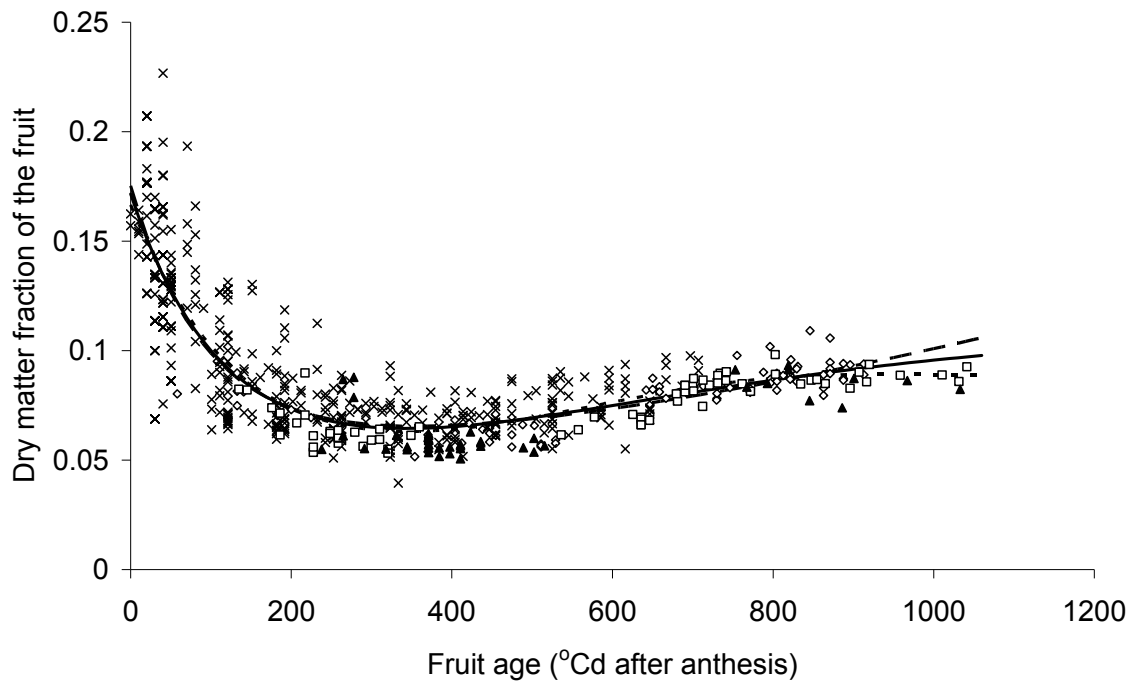


Fig. 3.1 Dry matter fraction of the fruit as function of fruit age (in degree-days) in the temperature experiment. Symbols represent the measurements, \diamond 18°C, \times 20°C, \square 21°C, and \blacktriangle 24°C, the lines represent the fit of specific function, — Ricker function, --- Polynomial function, - - - Bi-exponential function

Sigmoid functions

Should sigmoid functions be fitted on fresh or dry weight growth?

The ultimate objective is to obtain a function which can describe the dry weight growth of a fruit in relation to its age, and if relevant, in relation to temperature and cultivar. However, when the measurements of fruit dimensions were converted into fresh and subsequently into dry weight, dry weight did not have a clear plateau at the end (Fig. 3.3 and 3.4). Fruit fresh weight showed a sigmoid shape with a clear plateau in the end (Fig. 3.5 and 3.6). The increase in the dry matter fraction of the fruit had not yet ceased at the moment of harvest (Fig. 3.1 and 3.2). This implies that the fruit dry weight was still increasing at the moment of harvest. The sigmoid functions were therefore not fitted on estimated dry weight, but on estimated fresh weight.

Table 3.3 AICs for the functions relating fraction dry matter of the fruit to fruit age in the temperature and cultivar experiments.

Experiment	Function	AIC
Temperature	Polynomial	-2830
	Bi-exponential	-2825
	Ricker	-2831
Cultivar	Polynomial	-4663
	Bi-exponential	-4500
	Ricker	-4562

Sigmoid curves fitted on fresh weight

Fruit growth as a function of temperature sum was nearly similar at different temperatures, although the initial increase in fruit fresh weight was slightly slower and the maximum growth rate was reached later at 24°C than at either 18°C or 21°C (Fig. 3.5). The fruit growth curves of the six cultivars were very different (Fig. 3.6). The final fruit weight differed among cultivars, as well as the rate of increase and the shape of the curve.

Nearly all parameters indicating goodness of fit indicated the Richards function as the best model in both experiments (Table 3.4). Next in order were the Gompertz, Logistic and beta growth function. Graphically comparing data and fitted average curves revealed that the Logistic and the beta growth functions overestimated the data in the first part of the growth (Fig. 3.5 and 3.6). The Gompertz and Richard functions had a tendency to underestimate the first part of the growth curve.

Parameters w_{max} and k were not influenced by temperature, but they were influenced by cultivar (Table 3.5). The values of w_{max} and k were similar for cultivars ‘Fireflame’ and ‘Medina’ in all functions, but all other cultivars had different values for w_{max} and k . However, there was no correlation between the values of w_{max} and k .

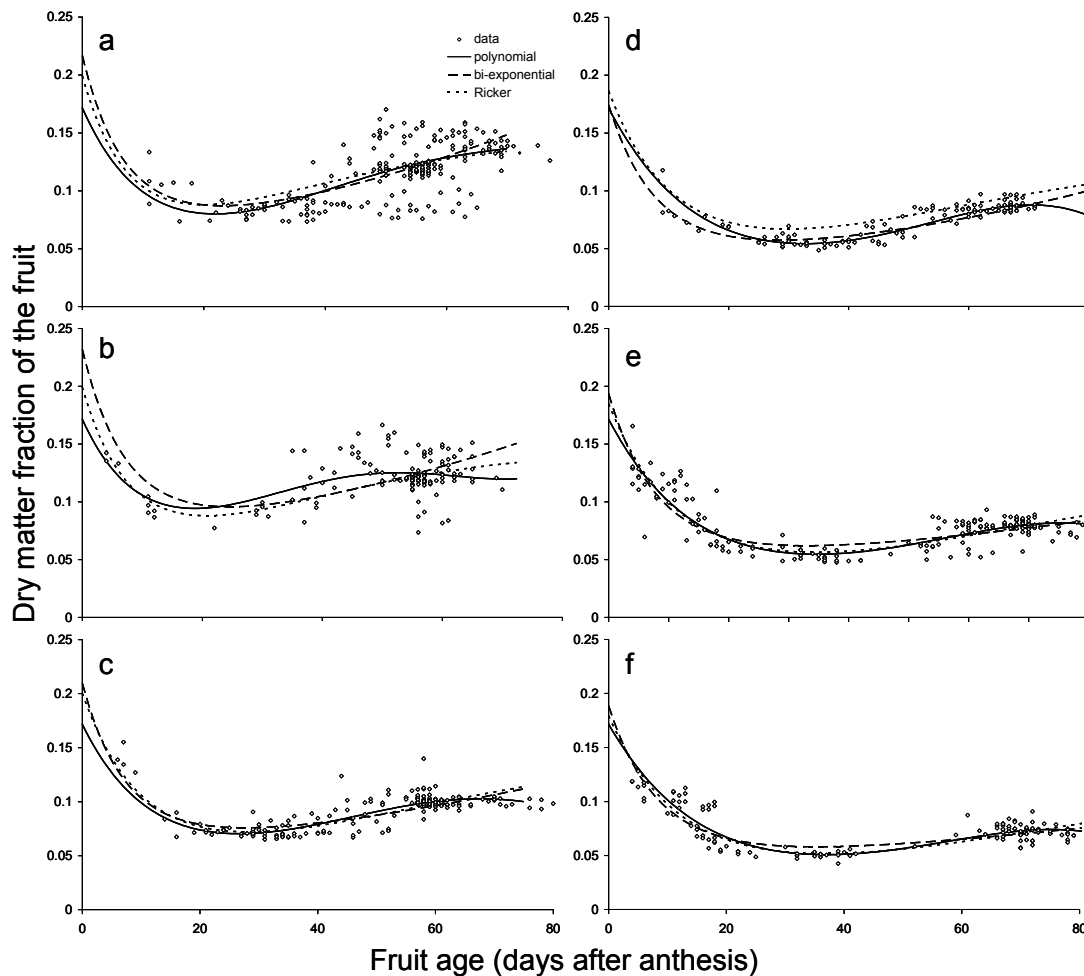


Fig. 3.2 Dry matter fraction of the fruit as function of fruit age (in days) for the six different cultivars. Symbols represent measurements, lines represent the different fitted functions. a) ‘Medina’, b) ‘Fireflame’, c) ‘Furila’, d) ‘Gepetto’, e) ‘Nazar’, f) ‘Funky’.

The shape parameter ν in the Richards function was affected by temperature (lower value as temperature increased) as well as cultivar. For all treatment levels, the values for ν were smaller than one. The value for ν was close to zero at 24°C and for the cultivar ‘Gepetto’, implying that the Richards function was nearly equal to the Gompertz function. Parameter t_m had a value of around one third of the fruit growth duration, with the Logistic and beta growth function estimating higher t_m values than the Gompertz and Richard functions.

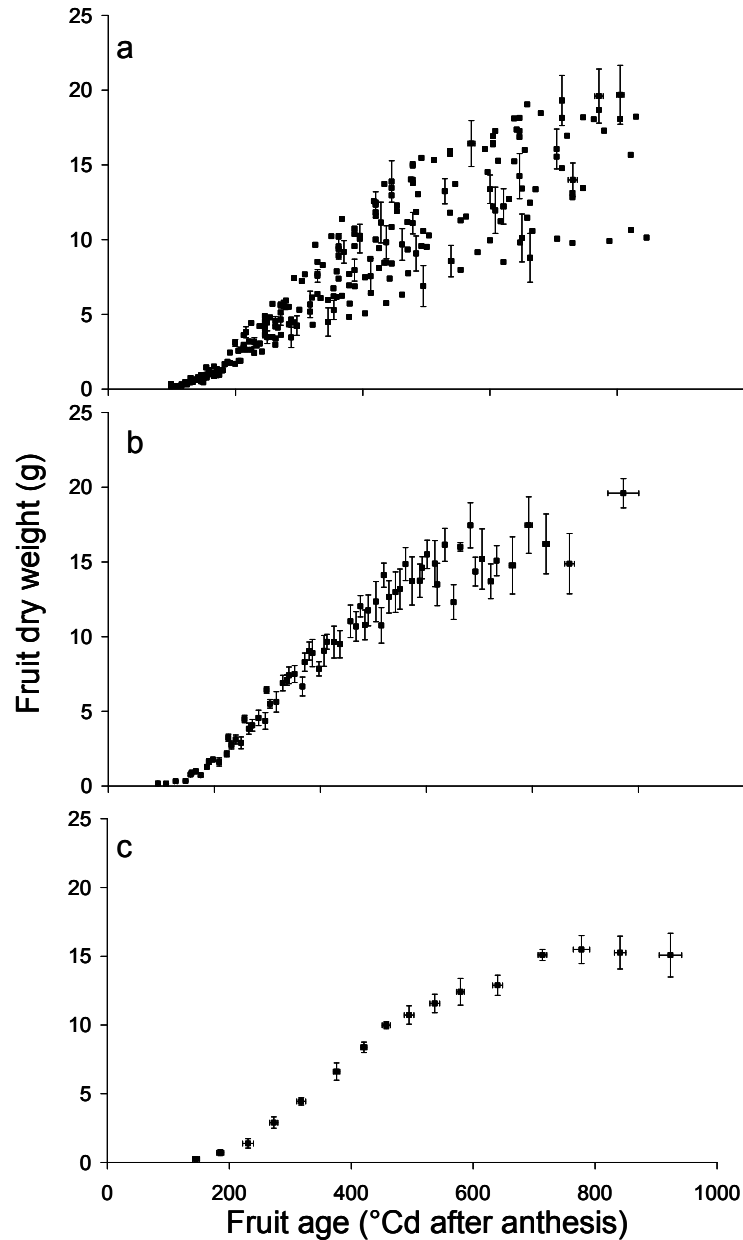


Fig. 3.3 Dry weight growth in the temperature experiment. Symbols represent the average over 5 data points. The Ricker function was used to calculate the fraction dry matter of the fruit as a function of fruit age. Error bars represent the standard errors of the mean when larger than the symbol. a) 18°C, b) 21°C, c) 24°C

Table 3.4 Measures of goodness of fit for the fits of four sigmoid curves on fruit growth data of the temperature and cultivar experiments. The R^2 is given for the regression of fitted values against observed values. The mean deviation (MD) and root mean square error (RMSE) are given for the difference of predicted minus observed values.

Experiment	Function	Log-likelihood	AIC	R^2	MD	RMSE
Temperature	Logistic	-2388	4793	0.9968	0.512	7.751
	Gompertz	-2377	4770	0.9975	-1.156	7.035
	beta growth	-2401	4824	0.9977	0.913	8.260
	Richards	-2358	4743	0.9964	-0.421	6.610
Cultivar	Logistic	-12097	24236	0.9942	0.601	5.152
	Gompertz	-11616	23273	0.9965	0.030	3.963
	beta growth	-12424	24874	0.9898	-1.021	6.797
	Richards	-11526	23103	0.9964	-0.050	3.849

In the temperature experiment, the time t_m at which the maximum growth rate was reached, appeared to be different for the different temperatures. However, adding temperature dependency to this parameter did not improve the fit ($P=0.63$ in the Gompertz function and $P=0.75$ in the Richards function) or the fit did not converge (Logistic and beta growth function). In the beta growth function, only w_{max} changed with temperature and cultivar. Parameter t_e was estimated around two thirds of the average growth period. A first-order autocorrelation between the data points significantly improved the fits of all functions in the temperature experiment. In the cultivar experiment, this was significant in the Logistic and Beta functions.

Discussion

Fitting a sigmoid curve on the dry weight increase of fruits was not appropriate, as a sigmoid shape with a clear plateau was not observed (Fig. 3.3 and 3.4). Data from literature also indicate the absence of a clear plateau when dry weight increase is plotted against time, e.g. for cucumber (Marcelis, 1992b), sweet pepper (Barrera *et al.*, 2008) and tomato (Ho *et al.*, 1982/3). Fitting a sigmoid curve on dry weight growth would therefore cause a relatively large uncertainty in the estimation of w_{max} . The parameter w_{max} , the final fruit weight, is an important indicator for fruit quality, and is also an important factor in modelling, as w_{max} has a large impact on e.g. dry matter partitioning and fruit abortion. A good fit of w_{max} is therefore important. Besides, the first 200°Cd or 20 days were difficult to fit properly for all functions when fitted on dry weight (data not shown). Fruit fresh weight often stabilises before the fruit is harvested (Fig. 3.5 and 3.6; Nielsen *et al.*, 1991; Turhan *et al.*, 2006; Barrera *et al.*, 2008). Therefore, the sigmoid curves should be fitted to fresh weight over time. Average dry weight over time can be obtained by the product of the sigmoid function and the function describing the relationship between fruit dry matter content and fruit age.

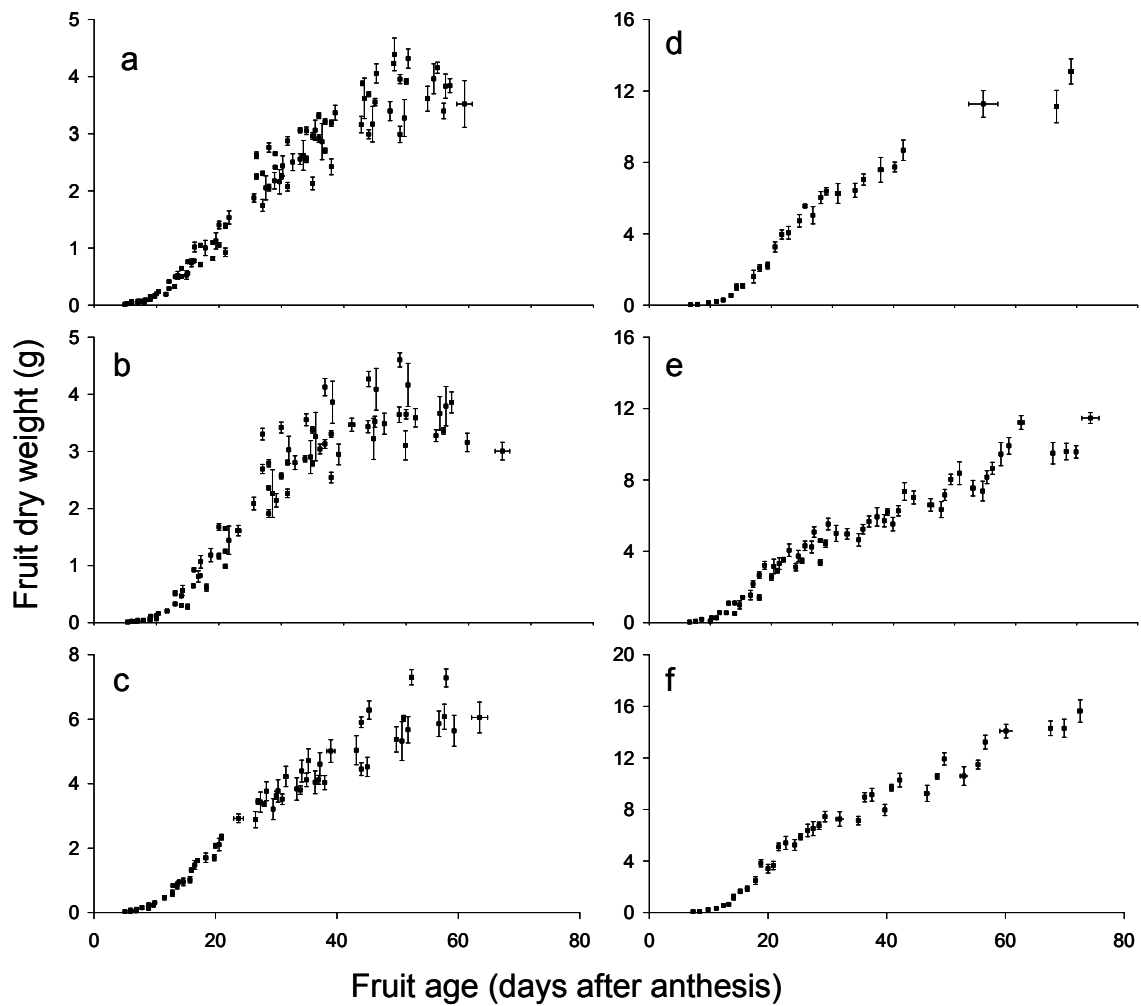


Fig. 3.4 Dry weight growth in the cultivar experiment. Symbols represent the average over 5 data points. The polynomial function was used to calculate the fraction dry matter of the fruit as a function of fruit age. Error bars represent the standard errors of the mean when larger than the symbol. a) 'Medina', b) 'Fireflame', c) 'Furila', d) 'Gepetto', e) 'Nazar', f) 'Funky'.

All tested functions described the fresh weight growth of sweet pepper rather well. In both experiments, the Richards function was found to have the best fit to the fresh weight against fruit age, which means that the extra flexibility provided by extra parameter ν was needed. The Gompertz function was nearly as good, and has the advantage of having one parameter less.

The large number of fruits used in this paper assures that the most appropriate function for sweet pepper is selected. All fruit growth curves were clearly not symmetric, and thus the Logistic function fitted worse than the Richards and Gompertz functions. The beta growth function was introduced by Yin *et al.* (2003) and has also been successfully used in other crops as well (Müller *et al.*, 2006; Condori *et al.*, 2008; Vocanson and Jeuffroy, 2008). This was the first time it was fitted on the growth of individual fruits. It fitted less well than the other functions. Parameter t_m and t_e were not affected by the treatments (Table 3.5). Variation in growth curves can therefore only be obtained with w_{max} , which was not sufficient to account for the different shapes of the measured growth curves.

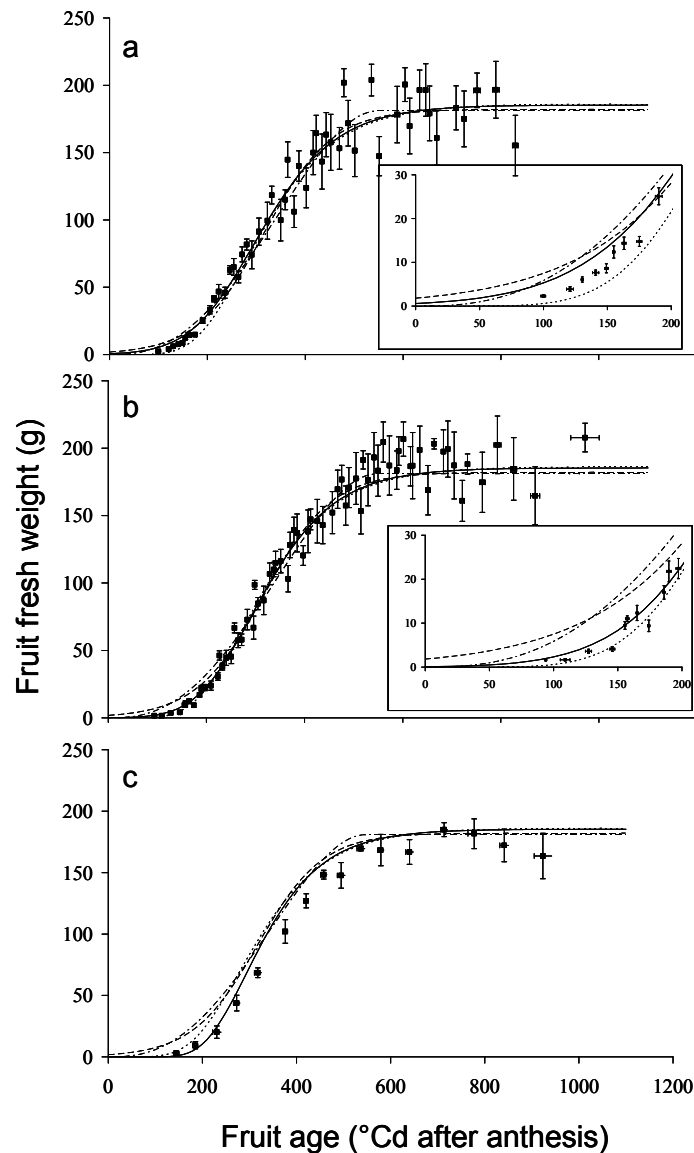


Fig. 3.5 Observed fresh weight growth and fitted functions of fruits in the temperature experiment. Inset magnifies the first 200°Cd of the curve when necessary. Symbols represent the average over 5 data points. Error bars represent the standard errors of the mean when larger than the symbol. Lines represent the average growth curve of the different functions. a) 18°C, b) 21°C, c) 24°C. — Richards function, --- Gompertz function, --- Logistic function and - - - - the Beta function.

Brown and Mayer (1988) fitted cumulative seed germination with six functions, three 3-parameter functions and three 4-parameter functions. The four-parameter functions, Weibull, Richards and Morgan-Mercer-Flodin, provided a better fit than the three-parameter functions, with the Weibull function fitting best. However, Avanza *et al.* (2008) reported that fitting 4-parameter functions on fruit growth of oranges was not successful. This might be due to the absence of the initial phase of slow growth. In our case, only one function with four parameters was used (the Richards function), which might create a bias towards this function.

Temperature only slightly influenced fruit growth, and only resulted in different parameter values between the temperatures when the Richards function was fitted. However, lower or higher temperatures than the ones presently used might affect fruit sizes. Fruit fresh weight in cucumber increased when the temperature was increased from 17.5°C to 30°C when fruits were grown solitarily (Marcelis and Baan Hofman-Eijer, 1993). With five fruits per plant, fruit fresh weight was higher at lower temperatures. Fruit dry weight in tomato decreased when the temperature was increased from 19°C to 23°C (De Koning, 1994). All stages of fruit growth were assumed to be equally sensitive to temperature. However, sensitivity to temperature changes with the development stage of the fruit. In hot pepper (Pagamas and Nawata, 2008) and tomato (Adams *et al.*, 2001), fruit growth duration was more sensitive to heat treatment given between 30 days after anthesis and harvest (pepper) or from 7 weeks after anthesis to harvest (tomato) than in other development stages. De Koning (1994) reported that young tomato fruits (in the first 30% of their development period) are sensitive to temperature changes. A higher sensitivity means that the fruit development would be faster than expected from the temperature sum, which might influence the final fruit size. Fruit growth differed largely between the cultivars; apart from the obvious difference in final size, the growth rate parameter k also differed between cultivars. Parameter t_m did not differ between the cultivars due to scaling of time to the fruit growth duration, but would differ between the cultivars when it is expressed in days (Barrera *et al.*, 2008).

González-Real *et al.* (2009) fitted a linear relationship between fruit dry weight and estimated fruit volume of sweet pepper. However, such a relationship is not appropriate as fruits with similar volumes can have different fruit dry weights, caused by variation in fruit age (which influences fruit dry matter content), and fruits of the same age can have different fruit volumes, due to different growth conditions. For our data, such a relationship would have an $R^2 \approx 0.7$, compared to $R^2 = 0.99$ for the relationship between fruit fresh weight and estimated fruit volume.

Two of the three relationships used for fitting fruit dry matter content to fruit age, the bi-exponential function and the polynomial function, had no asymptote, whereas the Ricker function had an asymptote c_R . The predicted value at fruit harvest for the Ricker function was about 25% (temperature) and 29% (cultivars) lower than the asymptote.

Table 3.5 Dependency of parameters on treatment levels, presence of random effects for each parameter and the auto-correlation between data points in the non-linear mixed model fit of the fruit fresh weight growth.

Experiment	Function	w_{max}	t_m	k^*	d	Random effects	correlation
Temperature	Logistic	—	—	—		w_{max}, t_m	0.70
	Gompertz	—	—	—		w_{max}, t_m	0.63
	beta growth	—	—	—		w_{max}, t_m, t_e	0.71
	Richards	—	—	—	temperature	w_{max}, t_m, k	0.57
Cultivar	Logistic	cultivar	—	cultivar		w_{max}, t_m, k	0.39
	Gompertz	cultivar	—	cultivar		w_{max}, t_m, k	n.s.
	beta growth	cultivar	—	—		w_{max}, t_m	0.60
	Richards	cultivar	—	cultivar	cultivar	w_{max}, t_m, k	n.s.

* in the beta function, column k indicates parameter t_e

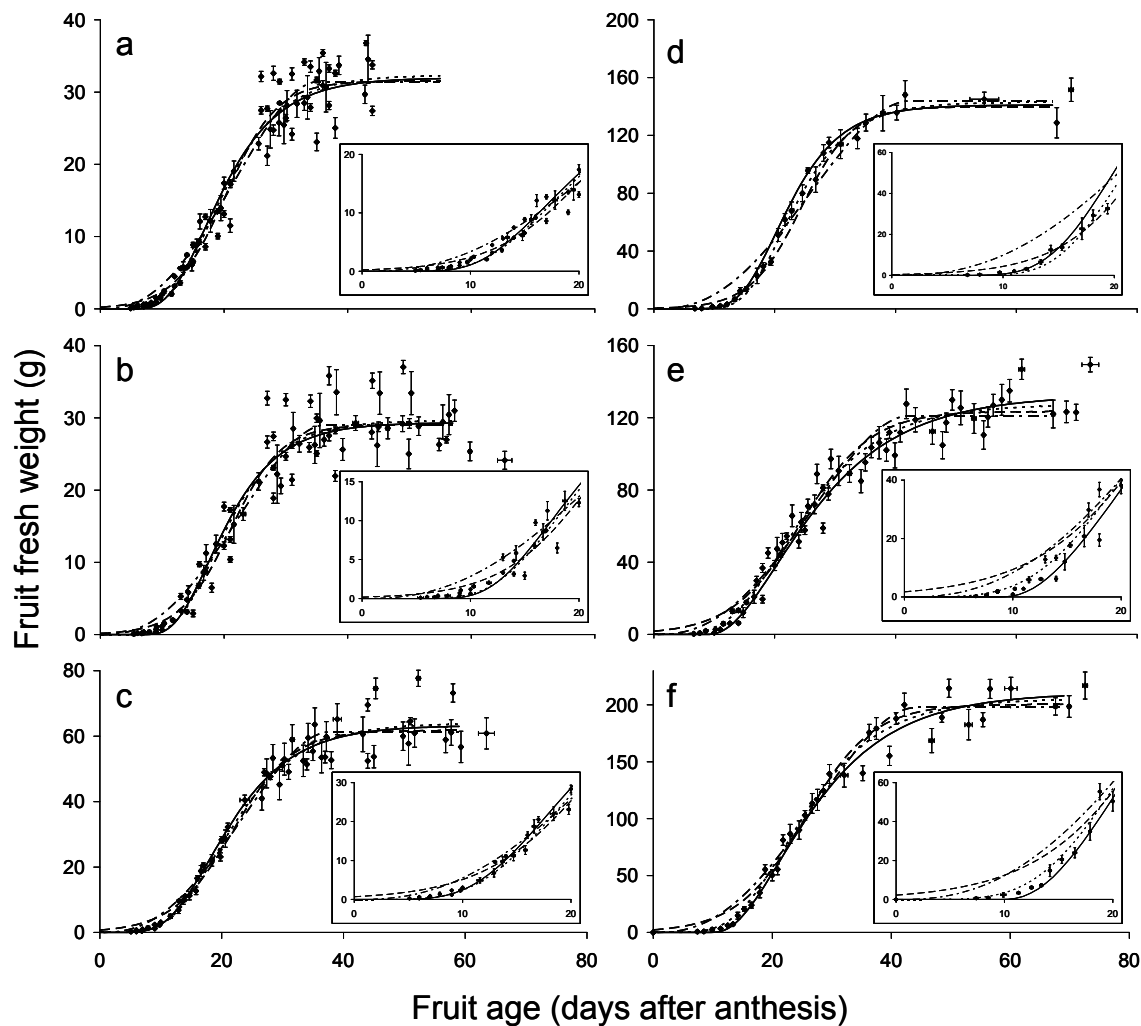


Fig. 3.6 Observed fresh weight growth and fitted functions of fruits in the cultivar experiment. Symbols represent the average over 10 data points. Error bars represent the standard errors of the mean when larger than the symbol. Lines represent the average growth curve of the different functions. a) 'Medina', b) 'Fireflame', c) 'Furila', d) 'Gepetto', e) 'Nazar', f) 'Funky'. — Richards function, --- Gompertz function, . . . Logistic function and - - - - the Beta function.

Cultivar had an influence on the relationship between fruit dry matter fraction and fruit age; the hot peppers ('Medina', 'Fireflame' and 'Furila') had a higher dry matter fraction than the sweet peppers. Dry matter fraction in the fruit shortly after flowering was not influenced by temperature, as was also the case for tomato (De Koning, 1994). Fruit load, season and salinity also influence dry matter fraction (De Koning, 1994). Ideally, these factors should also be incorporated in the function. In that case, non-linear regression analysis on a simple equation would not be sufficient to describe the relation.

This study shows that sigmoid function on fruit weight growth of sweet pepper should be fitted to fresh weight, and not to dry weight data, as fruit dry weight was still increasing at the time of harvest. To obtain dry weight, the function fitted to fresh weight could be multiplied by the function relating fruit dry matter content to fruit age to obtain the growth in dry weight. The Richards function is preferred for describing fruit growth of sweet pepper, but a Gompertz function was nearly as good.

Chapter 4

Fruit Set and Yield Patterns in Six *Capsicum* Cultivars

Abstract

Fruit set and yield patterns were studied in detail in six pepper cultivars. Fruit set differed largely between the cultivars: cultivars with small fruits (20-40 g fresh weight fruit⁻¹) showed higher fruit set (approximately 50%) than cultivars with large fruits (120-200 g fresh weight fruit⁻¹; 11% to 19%). The former showed continuous fruit set (4 to 5 fruits plant⁻¹ week⁻¹), while the latter showed fluctuations in fruit set. Fluctuations in weekly fruit set, expressed as the ratio between standard deviation of weekly fruit set and the mean of weekly fruit set (C.V.), were much lower for the cultivars with small fruits (0.44 to 0.49) than for the cultivars with large fruits (1.1 to 1.6). Fluctuations in weekly fruit yield varied between 0.51 to 0.77 for cultivars with small fruits and between 1.04 to 1.45 for cultivars with large fruits. Fluctuations in fruit yield were significantly positively correlated (Pearson R = 0.87) with fluctuations in fruit set. The correlation between fruit set and fruit yield patterns was highest with a lag time of 8 weeks for the cultivars with small fruits and 9 to 10 weeks for the cultivars with large fruits. This corresponds with the expected lag time based on the average fruit growth duration. The cultivars did produce the same amount of biomass, implying that source strength was more or less similar. Hence, differences in fruit set and fruit yield patterns between the cultivars were not the result of differences in source strength, and must therefore be related to differences in sink strength.

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Introduction

Flower and fruit abortion is a yield-limiting factor in many crops (Bacci *et al.*, 2006; Goldschmidt, 1999; Halbrecq *et al.*, 2005). Abortion can be caused by unfavorable conditions such as temperature stress (Guilioni *et al.*, 1997), low light conditions (low source strength; Aloni *et al.*, 1996), or limited pollination (Berjano *et al.*, 2006). Also under non-stressed conditions, sweet pepper flowers and young fruits abort due to competition for assimilates with fast growing fruits (strong sinks; Heuvelink *et al.*, 2004). Sweet pepper plants show an indeterminate growth pattern, which means that flowers are produced continuously. Together with the abortion of flowers and young fruits due to competition with fast growing fruits, this leads to alternating periods of high and low fruit set. These fluctuations in fruit set are believed to be the cause of cyclic fluctuations in fruit yield. Irregular fruit yield causes difficulties in the planning of activities throughout the production chain of sweet peppers and, as sweet pepper crops of different growers are synchronized, it causes fluctuations in the price of sweet peppers as well (Gottschall, 2001).

Sweet pepper cultivars are known to differ in fruit set percentage, especially under heat or light stress. Aloni *et al.* (1996) and Turner and Wien (1994a) observed differences in level of flower abortion between several cultivars, while Shifriss *et al.* (1994) found fruit set differences in different accessions under shading circumstances. However, fruit set was observed over a short time and patterns over time were not reported. Just a few examples of detailed patterns of fruit set within the plant are given, e.g. cucumber (Marcelis, 1992a), Salomon's seal (Gutián *et al.*, 2001) and maize (Reed and Singletary, 1989). Cultivar comparisons in fruit set are more abundant, e.g. pumpkin (Stapleton *et al.*, 2000), apricots (Alburquerque *et al.*, 2002), grapevine (Lebon *et al.*, 2004) and eggplant (Passam and Khah, 1992). Egli and Bruening (2006b) compared detailed fruit set patterns between two soybean cultivars. Detailed characterization of fruit set and yield patterns and investigation of reasons underlying cultivar differences in these patterns for pepper have not been done so far. As fluctuations in sweet pepper fruit set and fruit yield are a problem for the grower as well as for the rest of the production chain, analysis of this problem is necessary.

This Chapter aims at characterising and analysing differences between six pepper cultivars in fruit set and yield patterns. It was investigated whether fruit set fluctuations are indeed the cause of yield fluctuations. We elucidate on whether different fruit set patterns for different cultivars result from genotypic differences in source or sink strength.

Materials and Methods

Experimental setup

Seeds from six pepper cultivars (*Capsicum annuum* L.) differing in fruit size (20-205 g fresh weight; Table 4.1) were obtained from De Ruiter Seeds (the Netherlands). Seeds were sown on 1 Feb. in potting soil and after 10 days seedlings were transferred into rockwool cubes. On 30 March (when the first flower buds were appearing) plants were transferred to a 150 m² greenhouse compartment of a multispan Venlo-type greenhouse, in Wageningen, the Netherlands (52° N) and placed on rockwool slabs at a density of 3.8 plants m⁻². Heating set point was 18°C/15°C (day/night, length of day depended on sunset and sunrise) and ventilation set point was 1°C above heating set point. Temperature and relative humidity were recorded every five minutes using a commercial computer system (Hoogendoorn, Vlaardingen, the Netherlands).

Table 4.1 Cultivar names, their type, and their fresh fruit weight as indicated by the breeder (De Ruiter Seeds, the Netherlands).

Cultivar	Type	Fruit weight (g fw fruit ⁻¹)
‘Medina’	hot pepper	20
‘Fireflame’	hot pepper	20
‘Furila’	hot pepper	45
‘Gepetto’	pointed sweet pepper	135
‘Nazar’	block-shaped sweet pepper	140
‘Funky’	block-shaped sweet pepper	205

Realized average daily air temperature over the total growth period (30 March to 30 August) was $21.6 \pm 2.0^{\circ}\text{C}$ (mean \pm SD); average humidity was $77\% \pm 10\%$ (mean \pm SD). Irrigation was done with a standard nutrient solution for sweet pepper (BLGG, Naaldwijk, the Netherlands), with EC 2.5 dS m^{-1} and pH 5.5. No CO₂ enrichment was applied. Daily global radiation outside the greenhouse was obtained from an official weather station at about 300 m distance. The average global radiation outside the greenhouse during the total growth period was $16.3 \pm 5.6 \text{ MJ m}^{-2} \text{ d}^{-1}$ (mean \pm SD). Plants were pruned to two main stems, with the weakest branch of each dichotomous split pruned above the first flower and leaf.

A randomized complete block design was applied, with three blocks and six plots per block, each plot containing one cultivar. A plot consisted of 20 plants in a double row. Eight plants in each plot were used for destructive harvests. Guard plants were placed between plants used for destructive harvest and between the plots in the same row. In addition to the three blocks, there was an extra double row with six plots, each plot containing 10 plants of one cultivar. This double row was considered the fourth block in the analysis of fruit set, fruit yield, fruit weight and fruit growth duration.

Observations and destructive measurements

A total of 12 plants per cultivar was used for observations of flowering, abortion and fruit harvest dates. Six of these plants came from the extra double row (block 4), while the other six plants had been grown in the larger plots (two plants per block for each cultivar). Flowering and abortion dates were recorded six times a week. The week of fruit set was the week in which the flower reached anthesis. A fruit was defined to have been set if it eventually reached the harvestable stage or survived more than 10 (cultivars with small fruits) or 20 days (cultivars with large fruits). Completely red fruits were harvested and their fresh and dry weights were measured. This fruit harvest was done every Tuesday and Friday.

For each cultivar, five destructive harvests were done. The first destructive harvest occurred when the plants were placed on the slabs. At each of the four following harvests, six plants per cultivar (two from each block) were harvested. The harvest dates differed between the cultivars, as their elongation rates were different and cultivation had to be stopped when plants reached the top wire in the greenhouse. Leaf area was measured with a leaf area meter (Li-COR LI-3100, Lincoln, USA), and fresh and dry weight of leaves, stems and fruits of each plant were determined. Leaves and stems were dried for 12 h in a

ventilated oven at 105°C, while fruits were dried for two cycles of 12 h at this temperature. Roots were not measured. To each harvested plant, the weight of the already harvested fruits from that plant was added to obtain total plant weight and total fruit weight.

Calculation of desired variables

Percentage fruit set was calculated as the number of set fruits divided by the number of flowers times 100. In ‘Funky’, ‘Nazar’ and ‘Gepetto’, some buds aborted before flowering; they are included in the number of flowers in the calculation of percentage fruit set. Weekly fruit set (number of fruits plant⁻¹ week⁻¹) was calculated as the number of fruits which set within a calendar week. Weekly fruit yield was the total number of harvested fruits or the total harvested fruit weight of the two harvests in a calendar week. Fluctuations in weekly fruit set were quantified by the coefficient of variation, C.V. (= standard deviation / mean). C.V. for fruit set was calculated from the standard deviation of the weekly fruit set (number plant⁻¹ week⁻¹) and the mean of the weekly fruit set. Similar calculations were done for fruit yield, expressed in number of fruits (number of fruits plant⁻¹ week⁻¹) or in fruit weight (g dry weight plant⁻¹ week⁻¹). All C.V.s were calculated per plant for the 12 observational plants per cultivar. In order to be able to compare fluctuations in fruit set and yield between the cultivars, the C.V.s were calculated over the period in which all cultivars were present: week 15 to 28 for fruit set and week 24 to 29 for fruit yield. For the harvested fruits, fruit growth duration was calculated as the time between fruit set and fruit harvest. For the aborted fruits, the survival time was calculated as the time between flowering and abortion.

Statistical analyses

Percentage fruit set, C.V.s of fruit set and fruit yield, individual fruit weight (fresh and dry) and fruit growth duration were compared between the cultivars, based on the data until day 206 (week 29) of the 12 observational plants per cultivar. Individual fruit weight and fruit growth duration were averaged for each plant before analysis. Leaf area per plant, total fresh and dry plant weights and partitioning of dry matter into the fruits were compared between the cultivars in week 29 (days 200 to 206), based on the six harvested plants per cultivar in that week. Analyses were done with linear mixed models with cultivar as the fixed factor and cultivar nested within block as the error term. Fruit weight, the C.V.s and leaf area were allowed to have different standard deviations per cultivar in order to avoid trends in the residuals. Fruit set and dry matter partitioning into the fruits were analyzed as fractions. These fractions were arcsine-transformed before analysis to obtain normally distributed data (Sokal and Rohlf, 1994). If the cultivar effect was significant, differences between cultivars were tested with contrasts.

Timing of fruit abortion of the different cultivars was compared by means of Kaplan-Meijer survival analysis (Kleinbaum and Klein, 2005). In survival analysis, the times to an event of interest are analyzed. In this case, abortion of fruits is the event of interest and the survival time is the time between flowering and abortion. Fruits which do not abort but cannot be observed anymore (harvested fruits, unripe fruits at the end of the experiment) have a censored survival time: abortion was not observed before this time, but in theory it can still happen. The final value of the survival curve is the fraction of the total number of flowers that did not abort: harvested fruits or fruits that were still growing by the end of the observational period. This method in survival analysis produces a curve indicating which proportion of the fruits is still surviving at a certain time after anthesis.

With a log-rank test, survival curves were compared between the six cultivars for the period in which all of these were grown (up to day 206).

In order to compare fluctuations in fruit set with fluctuations in fruit yield (both in number plant⁻¹ week⁻¹) for each cultivar, the C.V.s for fruit set and fruit yield were correlated by using Pearson's correlation coefficient. In this case, fruit set in each plant was calculated from the first week of fruit set until the week when the last harvested fruits of the cultivar were set, and fruit yield from the first week of fruit yield in each plant until the last week of fruit yield of the cultivar. In order to determine whether the pattern in fruit yield is the lagged pattern of fruit set, correlations between the number of set fruits and the number of harvested fruits were made for different lag times.

All statistical analyses were done in R 2.6.0 (R Development Core Team, 2007).

Results

Fruit set

Fruit set percentage differed between the cultivars ($P < 0.001$, Table 4.2). Fruit set percentages of 'Fireflame' and 'Medina' differed, although the total number of set fruits was similar, caused by a higher number of flowers produced in 'Fireflame' plants (data not shown). Average weekly fruit set was higher for the small-fruited cultivars 'Medina', 'Fireflame' and 'Furila', compared to the large-fruited cultivars 'Gepetto', 'Nazar' and 'Funky' (Table 4.2, Fig. 4.1). The large-fruited cultivars showed a wave-like pattern with simultaneously timed peaks around weeks 15 and 16, 21, 24 and 30 (intervals of three to six weeks). The small-fruited cultivars also showed waves in fruit set, but for these the period appeared to be only two to three weeks. Fruit set started one week later for the large-fruited cultivars than for the small-fruited ones. Average weekly fruit set decreased with increasing fruit size, while the extent of the fluctuations (C.V.) increased with increasing fruit size (Table 4.2). The survival curves (fraction non-aborted fruits after anthesis) (Fig. 4.2) showed an initial decrease in the survival of the young fruits, but this leveled off earlier for the small-fruited cultivars (15 days after anthesis) than for the large-fruited cultivars (25 days after anthesis). Survival of the large-fruited cultivars ('Funky', 'Nazar' and 'Gepetto') started below one, because part of the buds aborted before anthesis.

Table 4.2 Fruit set, number of set and harvested fruits, and fruit yield are given for the six cultivars, together with their coefficient of variation (CV = SD/average). Fruit set was based on data up to week 28, weekly fruit set on data between weeks 15 and 28, fruit yield was based on data from weeks 24 to 29.

Cultivar	Fruit set (%)	Fruit set (number plant ⁻¹ week ⁻¹)		Fruit yield (g dw plant ⁻¹ week ⁻¹)		average	CV
		average	CV	average	CV		
'Medina'	59 d*	4.99 d	0.45 a	3.88 d	0.55 a	9.6 b	0.51 a
'Fireflame'	52 c	4.87 d	0.49 a	4.61 e	0.58 a	11.3 b	0.58 a
'Furila'	52 c	3.82 c	0.44 a	2.99 c	0.74 b	11.4 b	0.77 b
'Gepetto'	19 b	1.36 b	1.09 b	1.26 b	1.06 c	13.6 c	1.04 c
'Nazar'	19 b	1.26 b	1.19 b	1.21 b	1.37 d	10.7 b	1.42 d
'Funky'	11 a	0.69 a	1.54 c	0.56 a	1.39 d	7.4 a	1.45 d

* different letters within the same column indicate significant differences ($\alpha = 0.05$) obtained with contrasts (n = 12).

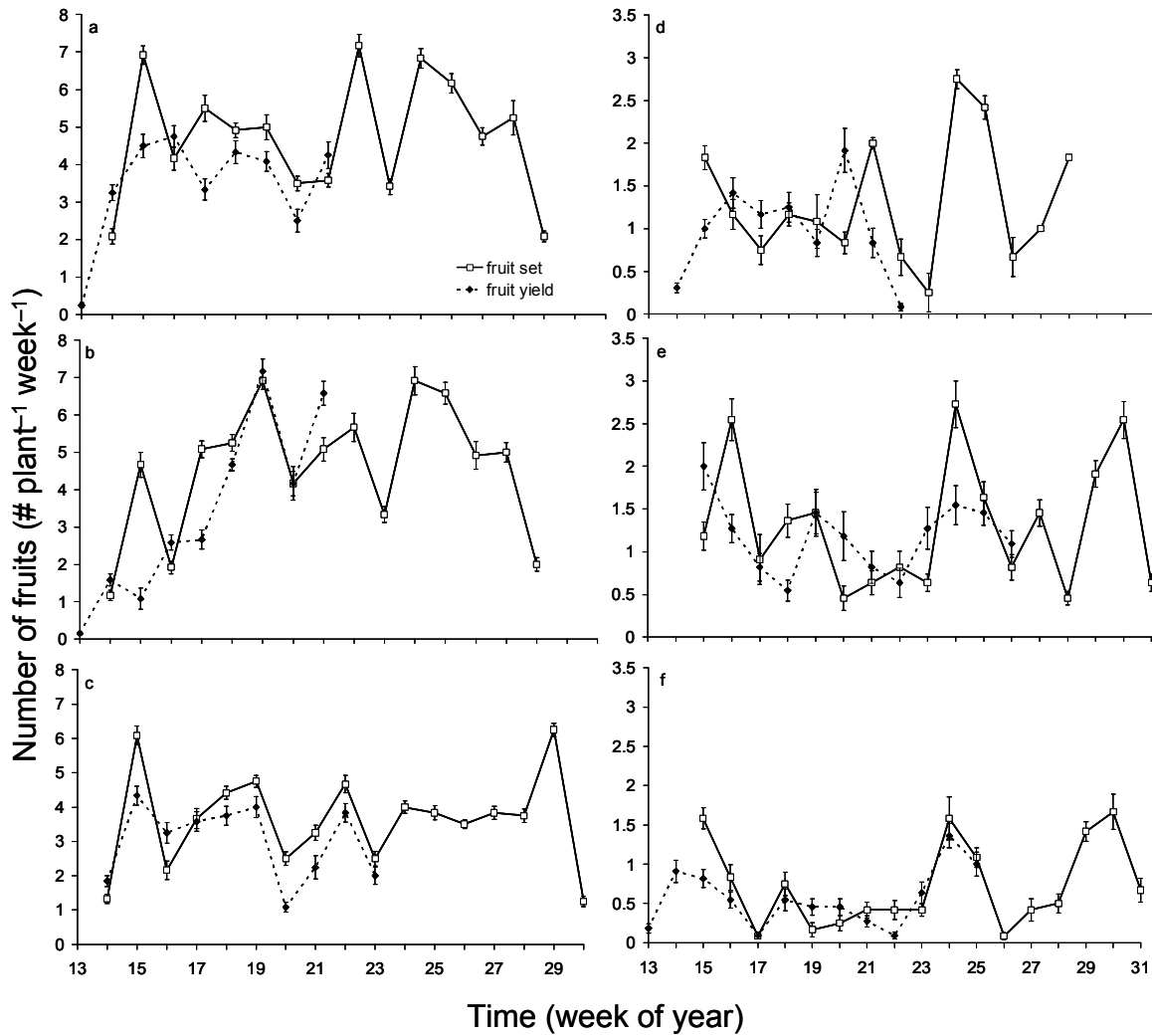


Fig. 4.1 Patterns of weekly fruit set (open symbols and continuous line) and weekly fruit yield (closed symbols and dashed line) (both in number plant⁻¹ week⁻¹) for the six cultivars. a) 'Medina', b) 'Fireflake', c) 'Furila', d) 'Gepetto', e) 'Nazar' and f) 'Funky'. In order to compare time patterns, the times at which fruits were harvested were brought forward 8 weeks (a, b and c), 9 weeks (d and e) or 10 weeks (f). Error bars indicate standard error (n = 12).

Survival curves of 'Fireflake' and 'Furila' were not significantly different from each other ($P=0.11$), as were the curves of 'Gepetto' and 'Nazar' ($P=0.99$). All other survival curves were significantly different ($P<0.001$).

Fruit yield

The quantity and timing of the number of harvested fruits differed between the cultivars (Fig. 4.1). After an initial increase, a considerable number of fruits was harvested weekly for the small-fruited cultivars 'Medina', 'Fireflake' and 'Furila'. Fruit harvest for the large-fruited cultivars 'Nazar', 'Gepetto' and 'Funky' started two weeks later (Fig. 4.1) and the number of harvested fruits per plant was lower in these cultivars than for the cultivars with small fruits (Table 4.2, Fig. 4.1). While the numbers harvested per plant per week differed largely between the cultivars, the harvested dry weight per plant per week was much more similar (Table 4.2). Dry weight of harvested 'Fireflake' fruits increased up to week 27,

while ‘Medina’ and ‘Furila’ had a more regular fruit yield (Fig. 4.3a). Fluctuations in harvested dry weight of the large-fruited cultivars were clearer than in number of harvested fruits (Fig. 4.1 and 4.3b), also reflected in a higher C.V. for the harvested dry weight (Table 4.2). Three peaks in fruit yield were registered for ‘Funky’ and ‘Nazar’. The first two peaks were almost at the same time, but the last one was one week earlier for ‘Nazar’ than for ‘Funky’. Fluctuations in fruit yield in cultivar ‘Gepetto’ had a different periodicity than for ‘Nazar’ and ‘Funky’ (Fig. 4.1 and 4.3b).

The coefficient of variation (C.V.) for yield in weeks 24-29 (number plant⁻¹ week⁻¹ and g dw plant⁻¹ week⁻¹) was significantly higher when fruit size was higher (Table 4.2). The range of C.V. values between the cultivars was smaller for fruit yield than for fruit set.

Relation between fruit set and fruit yield

The fluctuations in fruit yield (number plant⁻¹ week⁻¹) were strongly and positively correlated to fluctuations in fruit set (Pearson $R = 0.87$, $P < 0.001$, Fig. 4.4). When the average fruit growth durations in days (Table 4.3) were converted to weeks, the peak in yield of the cultivars with large fruits was expected to be 9, 9.4 and 9.8 weeks after fruit set for ‘Gepetto’, ‘Nazar’ and ‘Funky’. This was confirmed by correlations between fruit set and yield (both per plant) with a range of lag times. Most plants of these cultivars had a significant correlation when the lag was 9 or 10 weeks (Table 4.4), although weaker correlations appeared at week 8 and some plants had no correlation between fruit set and fruit yield at any lag time. Correlations were on average 0.79. The correlation was less clear when average fluctuations of fruit set and fruit yield were compared graphically (Fig. 4.1d-f). For the small-fruited cultivars, correlation between number of set fruits and number of harvested fruits was highest around week 8, the week expected based on average fruit growth duration, but the number of plants with a significant correlation was low. The graphic comparison of fruit set and fruit yield in these cultivars revealed that the average patterns showed strong similarities (Fig. 4.1a-c).

Table 4.3 Average individual fruit fresh and dry weights of harvested fruits, fruit growth duration, and plant cumulative dry weight, fresh weight, leaf area and fraction dry matter partitioned to the fruits up to week 29 for the six pepper cultivars.

Cultivar	Fruit weight		Fruit growth duration (days)	Plant weight		Leaf area (cm ² plant ⁻¹)	Dry matter in fruits (%)
	fresh (g fruit ⁻¹)	dry (g fruit ⁻¹)		dry (g plant ⁻¹)	fresh (g plant ⁻¹)		
‘Medina’	19 a*	2.4 a	57 a	220 a	1640 a	7525 a	52 d
‘Fireflame’	18 a	2.3 a	58 a	258 a	1806 a	7865 a	49 cd
‘Furila’	36 b	3.6 b	58 a	258 a	2244 b	10370 b	52 d
‘Gepetto’	129 d	10.8 d	63 b	274 a	2528 c	15192 c	35 a
‘Nazar’	119 c	8.9 c	66 c	253 a	2864 d	15282 c	47 c
‘Funky’	199 e	13.6 e	69 d	244 a	2623 cd	14440 c	40 b

* different letters within the same column indicate significant differences ($\alpha = 0.05$) obtained with contrasts ($n = 12$ for fruit characteristics, $n = 6$ for plant characteristics).

Fruit characteristics

As expected, average individual fruit fresh and dry weights of the cultivars differed from each other ($P < 0.001$, Table 4.3); only the cultivars ‘Medina’ and ‘Fireflame’ were not significantly different in fruit fresh and dry weights. There was no change in average fruit weight during the experiment (data not shown). Fruit growth duration differed significantly between the cultivars ($P < 0.001$, Table 4.3). ‘Medina’, ‘Fireflame’ and ‘Furila’ showed the same fruit growth duration, while ‘Nazar’, ‘Gepetto’ and ‘Funky’ had significantly longer fruit growth durations.

Total plant growth and development

At least up to day 180, there was no clear difference in dry matter production between the cultivars (Fig. 4.5a), but there was in leaf area (Fig. 4.5b). The cultivars with the smallest sized fruits (‘Medina’ and ‘Fireflame’) had a lower leaf area than ‘Furila’, which in turn had a lower leaf area than the three bigger sized cultivars. In week 29 (days 200 to 206), the differences between the cultivars were not significant for total plant dry weight ($P = 0.11$, Table 4.3), but they were for leaf area and total plant fresh weight ($P < 0.001$, Table 4.3). The small-fruited cultivars had a lower plant fresh weight. Fraction dry weight partitioned into the fruits up to week 29 was also significantly different between the cultivars ($P < 0.001$; Table 4.3). For ‘Medina’, ‘Fireflame’, ‘Furila’ and ‘Nazar’ dry matter partitioning to the fruits was approximately 50%, while ‘Gepetto’ and ‘Funky’ showed a lower partitioning to the fruits (35% to 40%). This difference was consistent during the whole cultivation period.

Discussion

Characterising and analysing cultivar differences in fruit set and yield patterns and relating fruit yield to fruit set patterns was the objective of this study. Small-fruited cultivars have much smaller relative fluctuations in fruit set and fruit yield than large-fruited cultivars. Both fruit set and yield patterns showed fluctuations, and the extent of the fluctuations in fruit yield was strongly positively correlated to fluctuations in fruit set (Fig. 4.4). The expected lag time between fruit set and fruit yield, i.e. the average growth duration (8 weeks for the small-fruited and 9 to 10 weeks for the large-fruited cultivars), was confirmed as well (Fig. 4.1, Table 4.4). Hence, fluctuations in fruit yield are indeed primarily caused by fluctuations in fruit set.

Table 4.4 Number of plants with a significant positive correlation between fruit set and fruit yield. The time series of weekly number of set fruits and the time series of weekly number of harvested fruits were correlated at a given lag time (weeks) ($n = 12$).

Lag time (weeks)	6	7	8	9	10	11	12
‘Medina’	0	0	3	1	0	0	0
‘Fireflame’	1	1	6	2	0	0	0
‘Furila’	0	0	5	0	0	1	0
‘Gepetto’	0	0	1	3	1	0	0
‘Nazar’	0	0	1	4	2	0	0
‘Funky’	0	0	0	1	6	1	0

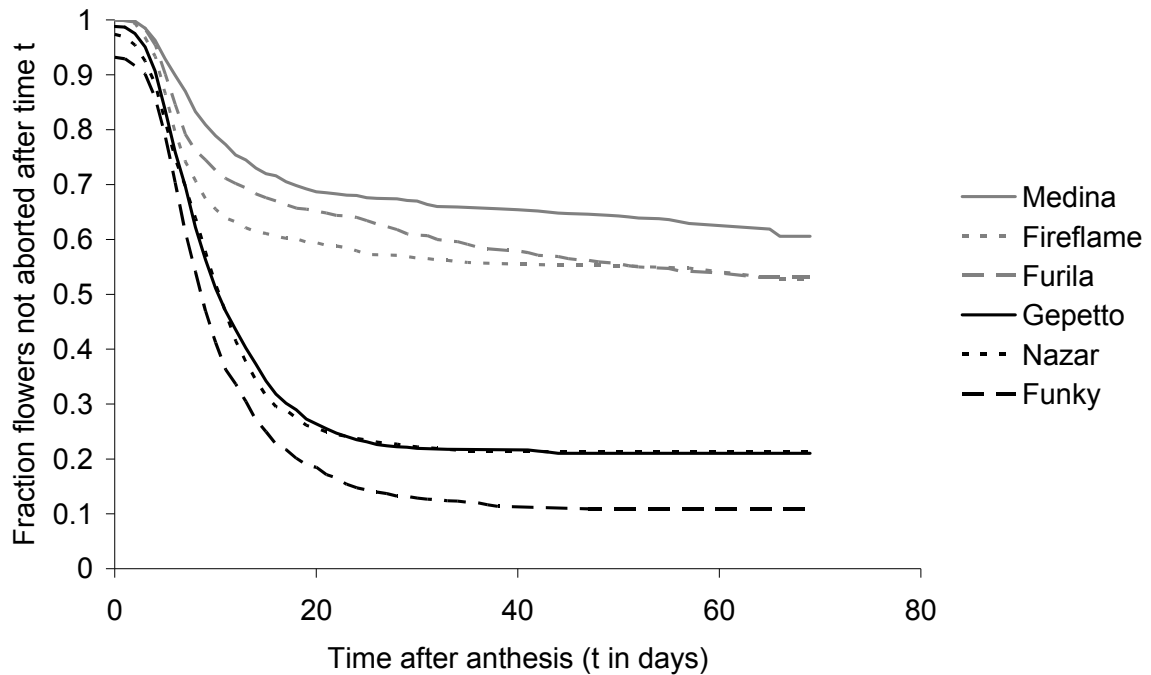


Fig. 4.2 Survival curves of fruits for the six cultivars. The survival time of a fruit is the time from flowering to abscission. Lines represent the population averages of survival times. Survival curves of ‘Gepetto’, ‘Nazar’ and ‘Funky’ start below one, because a number of the buds aborted before flowering. Survival is based on data from the start of the experiment, day 89, up to day 206.

The extent of the fluctuations in fruit yield were not the same as the fluctuations in fruit set: the small-fruited cultivars and ‘Nazar’ had more fluctuation in fruit yield than in fruit set, while fluctuations in ‘Funky’ were slightly smaller in fruit yield than in fruit set. This could be due to small variations in fruit growth period and/or variations in harvest stage. With regard to the timing of fruit abortion, this was related to the fruit growth duration, as the period after flowering during which fruits could abort is longer for the cultivars with longer fruit growth duration (Fig. 4.2).

Biomass production over time was similar for all cultivars. When biomass production is taken as a measure for source strength, it implies that the differences in fruit set between the cultivars are not caused by differences in source strength. Day-to-day variations in radiation resulted in similar changes in source strength for all the cultivars. Inspection of weekly fruit set and weekly average radiation revealed no clear influence of average radiation on fruit set. Fruit set in sweet pepper is known to depend on competition from fast-growing fruits (Marcelis *et al.*, 2004). As more fruits set, sink strength increases and reduces fruit set of subsequent flowers. When the fruits are nearly ripe, new flowers can set fruit (Heuvelink *et al.*, 2004). This resulted in fluctuations in fruit set for the cultivars with large fruits (‘Gepetto’, ‘Nazar’ and ‘Funky’), but not for the cultivars with small fruits. Fluctuations in fruit set in the cultivars with small fruits were relatively low (low C.V.), despite the fact that fast-growing fruits were always present. Cultivars with small fruits producing higher numbers of fruits than cultivars with large fruits were also

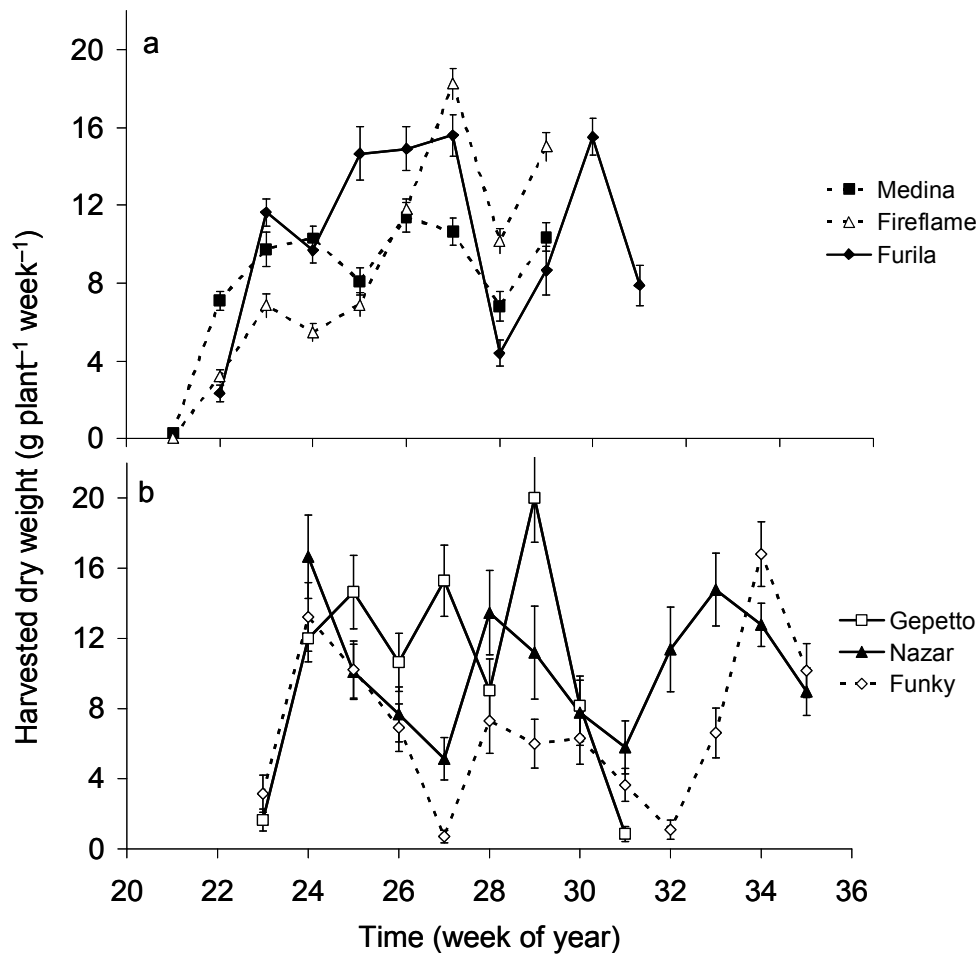


Fig. 4.3 Weekly fruit yield (g dw plant⁻¹ week⁻¹) for the cultivars with small fruits (a) and large fruits (b). Error bars indicate standard error (n = 12).

observed in cucumber (Jasso-Chaverria *et al.*, 2005) and pumpkin (Stapleton *et al.*, 2000). The larger fruit weight in cultivars with large fruits was not primarily caused by a difference in fruit growth duration, as this was small compared to the differences in fruit weight (Table 4.3). Differences in fruit size must therefore be caused by differences in fruit growth rate, which can differ between cultivars (Amorós *et al.*, 2003; Cheng and Breen, 1992; Zhang *et al.*, 2005). Higher fruit size and fruit growth rate can be related to higher fruit sink strength (potential fruit growth rate, Marcelis, 1996), but the fruit sink strength of the different cultivars is not yet known. As source strength for the different cultivars was more or less similar, differences in fruit sink strength between the cultivars are the most likely explanation for different fruit set patterns in the cultivars. Lower sink strength per fruit would imply that more fruits can set, which is seen in the present experiment. The similarity in total biomass production is remarkable, since leaf area vastly differed between the cultivars. Differences in plant architecture and plant height between the cultivars could explain this.

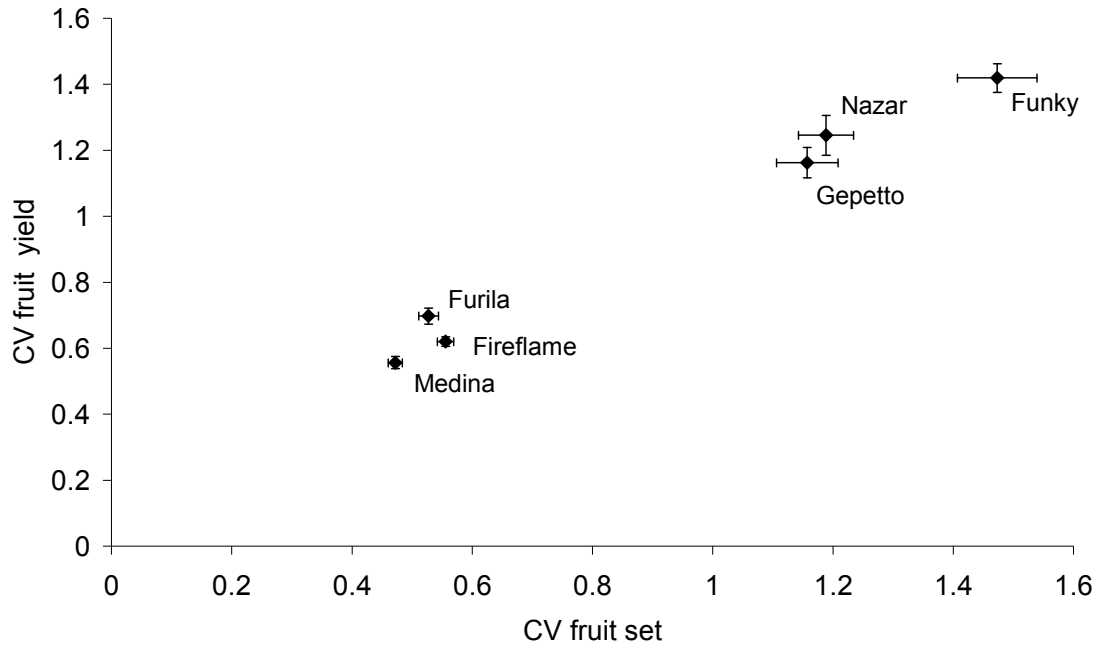


Fig. 4.4 Average coefficient of variation (C.V.) for weekly fruit yield plotted against average C.V. for weekly fruit set (both in number plant⁻¹ week⁻¹) for the six cultivars. Error bars indicate standard error (n = 12).

The cultivars with small fruits had a more open canopy, which is favorable for light penetration and light interception lower in the canopy (Sassenrath-Cole, 1995). The cultivars with small fruits grew also much stronger in height, thereby causing shade for the adjacent cultivars with large fruits. This resulted in higher light interception for the former than what would have occurred in a homogeneous crop, while the latter received less light than in a homogeneous crop. In a homogeneous crop, fruit set would have been higher in the large-fruited cultivar and lower in the small-fruited cultivars.

In conclusion, fruit set in *Capsicum* cultivars did differ in the percentage fruit set, with higher fruit set in cultivars with small fruits resulting in a more homogeneous fruit set in time (less fluctuations). Fluctuations in fruit yield could be explained from the fluctuations in fruit set, as magnitude of both fluctuations were strongly positively correlated and the lag time between fruit set and fruit yield was as expected from the average fruit growth duration. Differences in fruit set patterns between cultivars were not caused by differences in source strength, but were probably the result of differences in fruit sink strength.

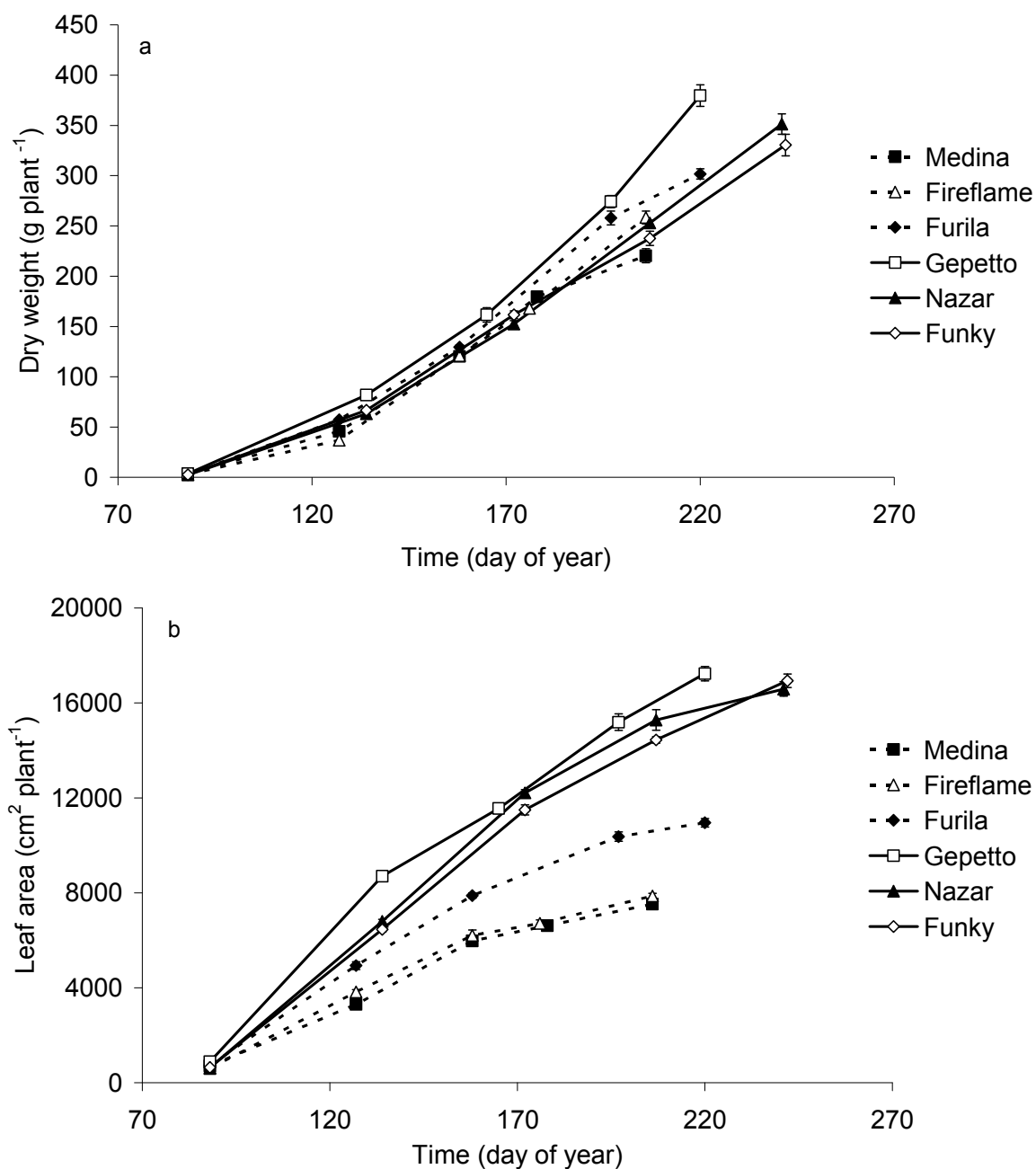


Fig. 4.5 Total above-ground biomass (a, g dw plant⁻¹) and leaf area (b, cm² plant⁻¹) for the six cultivars for each destructive harvest. Error bars indicate standard error (n = 6).

Chapter 5

Genetic differences in fruit set patterns are determined by differences in fruit sink strength and a source:sink threshold for fruit set

Abstract

Fruit set in indeterminate plant species largely depends on the balance between source and sink strength. Plants of these species show fluctuations in fruit set during the growing season. It was tested whether differences in fruit sink strength among the cultivars explained the differences in fruit set patterns. Capsicum annuum was chosen as a model plant. Six cultivars with differences in fruit set, fruit size and plant growth were evaluated in a greenhouse experiment. Fruit set patterns, generative and vegetative sink strength, source strength and source-sink ratio at fruit set were determined. Sink strength was quantified as potential growth rate. Fruit set was related to total fruit sink strength and source-sink ratio. The effect of the differences observed in the above-mentioned parameters on fruit set patterns was examined using a simple simulation model. Sink strengths of individual fruits differed greatly among cultivars. Week-to-week fruit set in large-fruited cultivars fluctuated due to large fluctuations in total fruit sink strength, but in small-fruited cultivars, total fruit sink strength and fruit set were relatively constant. Large variations in week-to-week fruit set were correlated with a low fruit set percentage. The source:sink threshold for fruit set was higher in large-fruited cultivars. Simulations showed that within the range of parameter values found in the experiment, fruit sink strength and source:sink threshold for fruit set had the largest impact on fruit set: an increase in these parameters decreased the average percentage fruit set and increased the variations in weekly fruit set. Both were needed to explain the fruit set patterns observed. The differences observed in source strength, vegetative sink strength and flower formation rate could not explain the differences observed in fruit set.

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Introduction

Indeterminate crops can show cyclical patterns in fruit set (Schapendonk and Brouwer, 1984; Passam and Khah, 1992; Heuvelink *et al.*, 2004); periods with high fruit set alternate with periods of low fruit set. Different explanations have been proposed for these patterns: hormones exported by growing fruits may inhibit fruit set of new fruits (Bangerth, 1989), and competition for assimilates between rapid growing fruits and young fruits may cause abortion of young fruits (Bertin, 1995; Marcelis *et al.*, 2004). It has also been suggested that both explanations interact; a decrease in import of assimilates into the fruit might change hormone levels, leading to abscission (Aloni *et al.*, 1997; Marcelis *et al.*, 2004).

In the competition theory, key concepts are source and sink strength, representing the supply and demand for assimilates, respectively. The sink strength of a growing organ can be quantified as its potential growth rate (Marcelis, 1996) and depends on its developmental stage (Schapendonk and Brouwer, 1984; Marcelis and Baan Hofman-Eijer, 1995). The total sink strength of a plant comprises the sink strength of all the organs. The source strength is the supply of assimilates, originating from the photosynthesis. Assimilates are divided over the organs in proportion to their fractional contribution to the total sink strength (Marcelis, 1996). When the total sink strength is high, due to many growing fruits, flowers and young fruits are not able to compete for assimilates with the fast growing fruits and hence abort. High sink strength, caused by a high fruit load, has resulted in low fruit set in, for instance, sweet pepper (Marcelis *et al.*, 2004), tomato (Bertin, 1995) and cotton (Pettigrew, 1994).

As sink strength plays an important role in fruit set (see above), differences in the sink strength of an individual fruit are expected to result in different fruit set patterns. Sink strength of an individual fruit often shows a bell-shaped curve skewed to the right as a function of time after anthesis (Marcelis, 1993). Fruit sink strengths of different cultivars may differ in their maximum growth rate, the timing of the maximum growth rate, and fruit growth duration (time between anthesis until harvest ripe). For example, potential fruit weight of long-life, cherry and beefsteak tomato cultivars differed significantly (Bertin *et al.*, 1998), and in peach, fruit growth duration and maximum fruit growth rate of two cultivars differed, resulting in different potential fruit weights (Grossman and DeJong, 1995). However, cultivars may also differ in other properties such as source strength (which is affected by leaf area, plant architecture and photosynthetic characteristics), rate of flower formation and the vegetative sink strength. Source strength is also known to influence fruit set (Marcelis *et al.*, 2004). Simulation studies have shown that an increase in vegetative sink strength decreased the number of fruits (Marcelis, 1994).

In this study, an experiment was conducted to elucidate which factors determine differences in fruit set patterns. Fruit set was followed in detail in six *Capsicum* cultivars with different fruit sizes. We tested the hypothesis that differences in fruit set patterns between cultivars are due to differences in individual fruit sink strength, by combining experimental results with a simple simulation model.

Materials and methods

Experimental set-up

Six *Capsicum* cultivars with different potential fruit weights were used in the experiment. ‘Medina’ (20 g), ‘Fireflame’ (20 g) and ‘Furila’ (45 g) are hot pepper cultivars, ‘Gepetto’ (135 g) is a cultivar with pointed sweet peppers and ‘Nazar’ (140 g) and ‘Funky’ (205 g) produce block-type sweet peppers; values between brackets are the representative fruit fresh weights of each cultivar, as published by the seed company (De Ruiter seeds, the Netherlands). Plants were grown in a Venlo-type greenhouse compartment on rockwool substrate in Wageningen, the Netherlands (lat 52°N), from April until September at a density of 3.8 plants m⁻². Two stems per plant were retained. Average temperature was 21.6 ± 2.0°C (mean ± s.d.), recorded using a commercial computer system. Average daily global radiation was 16.3 ± 5.6 MJ m⁻² d⁻¹ (mean ± s.d.), and was recorded at an official weather station at 300m distance. The experiment was set up as a randomized complete block design, with three blocks and six plots per block, each plot containing one cultivar. A plot consisted of 20 plants in a double row. Eight plants in each plot were used for destructive harvests. Guard plants were placed between plants used for destructive harvest and between the plots in the same row. There was a fourth block, containing additional plants for observations on fruit set, fruit growth duration and fruit weight. It consisted of a double row with six plots, each plot containing 10 plants of one cultivar.

Measurements

Data on plant weight and leaf area were measured at five destructive harvests. Six plants per cultivar (two plants per block) were used in each destructive harvest. Leaf area was measured using the Li-COR measurement system (LI-3100, Lincoln, USA). Observations on flowering and fruit set were made six times a week on 12 plants per cultivar, six plants in the fourth block and the six plants of the last destructive harvest (2 per block). Red fruits were harvested twice a week. Results of plant weight, leaf area and yield patterns are given in Chapter 4 (Wubs *et al.*, 2009a).

Fruit sink strengths, quantified by the potential fruit growth rates, were obtained by non-destructive measurements on potentially growing fruits, as described by Marcelis and Baan Hofman-Eijer (1995). Potentially growing fruits were fruits growing with very low competition from other fruits, which could attain their potential fruit size. Conditions for potential fruit growth were created by tagging two flowers on a plant from which all fruits were removed. New flowers were removed weekly. Twice a week, length and diameter of the tagged fruits were measured to obtain fruit volume. Number of measured fruits ranged from 29 to 83 fruits per cultivar. To convert fruit volume into fruit dry weight, a relationship between volume and fresh weight, and a relationship between fruit age and dry matter fraction of the fruits had to be obtained. For the first relationship, fruit volume and fruit fresh weight of 178 to 341 randomly sampled fruits per cultivar were measured. For the second relationship, the ages and dry matter percentages of 114 to 229 fruits per cultivar were measured.

Data analysis

A fruit was considered to be set if it reached the harvestable stage or if it survived for more than 10 days in small-fruited cultivars or more than 20 days in large-fruited cultivars.

Percentage fruit set was calculated as the number of fruits set divided by the number of flowers times 100.

On the basis of the lengths and diameters of the potentially growing fruits, their volume was calculated assuming a cylindrical fruit shape. This was subsequently converted into fresh weight, using a linear regression fitted between volume and fresh weight (R^2 0.99). A Gompertz function (equation 5.1) was fitted through fresh weight over time (loglikelihood -11616).

$$w(t) = w_{\max} e^{-e^{-k(t-t_m)}} \quad \text{equation 5.1}$$

$w(t)$ is the weight at age t (d after anthesis), w_{\max} is upper asymptote of fruit weight (g), k represents the weighted mean relative growth rate and t_m the age (d) at maximum growth rate.

The Gompertz function was fitted through the data with non-linear mixed modelling. Non-linear mixed models take into account that the measurements on one fruit are grouped. A lower variation is assumed between the measurements of one fruit than between the measurements of different fruits. A mean and standard deviation were estimated for the three model parameters (w_{\max} , k , and t_m). The three parameter means were used to describe fruit growth.

As there was variation in final fruit size, the fitted value of w_{\max} was increased to reach the average weight of the largest 10% of the fruits. This required increasing w_{\max} by 30% for all cultivars.

A sum of two exponential functions was fitted through the data of fruit age and dry matter fraction (equation 5.2, loglikelihood 2265).

$$\text{fdm}(t) = a_s e^{(b_s t)} + c_s e^{(d_s t)} \quad \text{equation 5.2}$$

$\text{fdm}(t)$ is the fraction dry matter of the fruit at age t (days after anthesis) and a , b , c and d are parameters.

The sink strength (representing the potential growth rate in g dry matter) was the derivative of the product of the Gompertz function and the sum of two exponential functions. Potential fruit dry weight was calculated as the upper asymptote of the Gompertz function w_{\max} (g fw) multiplied by the fraction dry matter of the fruit at the average harvest time (= average fruit growth duration).

The correlation of potential fruit dry weight with the percentage fruit set and the variation in weekly fruit set was determined. Weekly fruit set was the number of fruits set per week. The variation in weekly fruit set was defined as the coefficient of variation, C.V., which is the standard deviation of weekly fruit set divided by the mean weekly fruit set. The variation in weekly fruit set was calculated using data from weeks 15 to 28. Correlations were quantified using Spearman R, which estimates the correlation between the ranks of two non-normal distributed variables. All analyses were done in R version 2.6.0 (R Development Core Team, 2007).

Fruit growth duration, maximum potential fruit growth rate and the ratio between actual and potential fruit weights were examined. Fruit growth duration was the time between flowering and harvest. It was calculated from fruits growing on the 12 plants used

for observing fruit set as well as potentially growing fruits, because there were no significant differences between these two groups (data not shown). The maximum in the potential fruit growth rate (g dm d^{-1}) was the top of the sink strength curve. The ratio of actual to potential fruit weight is a measure for the source-sink ratio: it represents the ratio of actual to potential growth of a fruit.

The sink strength of a set fruit was calculated for each day between flowering and harvest. The total fruit sink strength of a plant was calculated per day by accumulating the sink strength of all fruits which were present that day. The total fruit sink strength per cultivar was averaged over the 12 plants observed. Patterns of total fruit sink strength over time were compared between the cultivars and related to weekly fruit set for each cultivar.

Source strengths of the cultivars were calculated using the crop growth model INTKAM (Marcelis *et al.*, 2006) with measured leaf area index, radiation and temperature as input. Simulated dry matter production was calibrated on the total measured plant weight. Source strengths over time were comparable for all cultivars (see data on total plant growth in Wubs *et al.*, 2009a).

Vegetative sink strength was assumed to be constant in time. It was estimated by iteration using simulated source strength, calculated total fruit sink strength and observed dry matter partitioning into the fruits at the five destructive harvests. The total squared deviation between measured and calculated dry matter partitioning into the fruits for the five destructive harvests was minimised. The average deviation between estimated and realised partitioning was between 0.009 and 0.046.

Source-sink ratio was calculated based on total fruit sink strength, vegetative sink strength and source strength. Source-sink ratio was assumed to determine fruit set (Bertin, 1995). The average source-sink ratio at fruit set was calculated.

Simulation studies

A simple deterministic simulation model was developed in Scilab 4.1.2 (www.scilab.org) to study the effect of different parameters on fruit set. Source strength, vegetative sink strength and flower appearance rate were assumed to be constant. Source and sink strength define abortion and assimilate partitioning. Sink strength of a fruit was calculated using the first derivative of the Gompertz function. In the Gompertz function, the maximum growth rate was at one third of the fruit growth duration and potential fruit dry weight was the asymptote. Fruit set was regulated by the source-sink ratio: if the source-sink ratio at anthesis was above a certain threshold, the flower would set into a fruit. The realised growth rate of a fruit depended on its share in the total sink strength and on source strength. Model parameters were in the range of values found in the cultivars; the minimum and maximum value and, if applicable, an intermediate value (Table 5.1). Model equations are found in the appendix of Chapter 5.

Simulations were carried out for 200 days. For each simulation, the number of fruits set as well as the percentage fruit set, the variation in fruit set (C.V.), the average fruit weight of the fruits harvested and the ratio of actual to potential fruit weight were calculated. Variation in fruit set was based on ‘weekly’ numbers of fruits set, where ‘weekly’ fruit set was obtained by counting the numbers of fruits set every seven days.

To investigate the effect of a given parameter on the simulation output (sensitivity analysis), each of the six parameters was changed one-by-one while keeping the default value for the remaining parameters (Table 5.1). Next, the parameter values were changed

Table 5.1 Parameter values for the simulations. The first line gives the default parameter values. The second line gives the parameter values used in the sensitivity analysis. One parameter was changed, while keeping the other parameters at the default value. Small-fruited and large-fruited cultivar represent the parameter combinations for the simulations with realistic parameter combinations.

	Potential fruit weight (g dm)	Fruit growth duration (d)	Flower appearance rate (d^{-1})	Source strength (g dm d^{-1})	Vegetative sink strength (d dm d^{-1})	Threshold source-sink ratio for fruit set
Default values	8	60	0.5	2.4	13.9	0.5
Value(s) for sensitivity analysis	4, 18	57, 70	1	2.2, 2.6	1.6, 2.9	1
Small-fruited cultivar	4	57	1	2.2	1.9	0.55
Large-fruited cultivar	18	70	0.5	2.4	1.6	1.0

simultaneously, taking into account combinations observed in the cultivars (Table 5.1). These simulations resembled cultivars with small- and large-sized fruits.

Results

Experiment

Fruit sink strengths differed in maximum growth rate and fruit growth duration (Fig. 5.1; Table 5.2), although the differences in maximum growth rate were much larger than in fruit growth duration. Together, the differences in maximum growth rate and fruit growth duration resulted in different potential dry weights. Timing of the maximum growth rate differed slightly (Fig. 5.1), but was the same on a normalized scale, namely at one third of the fruit growth period. The cultivars showed different percentage fruit set (Fig. 5.2a) as well as different fruit set patterns in time (Fig. 5.3). The higher the potential fruit weight of a cultivar, the lower the percentage fruit set (Fig. 5.2a; Spearman $R = -0.93$, $P < 0.001$). A higher potential fruit weight increased the variation in weekly fruit set (higher C.V., Fig. 5.2b; Spearman $R = 0.80$, $P < 0.001$). The percentage fruit set and variation in fruit set correlated negatively (Spearman $R = -0.85$, $P < 0.001$).

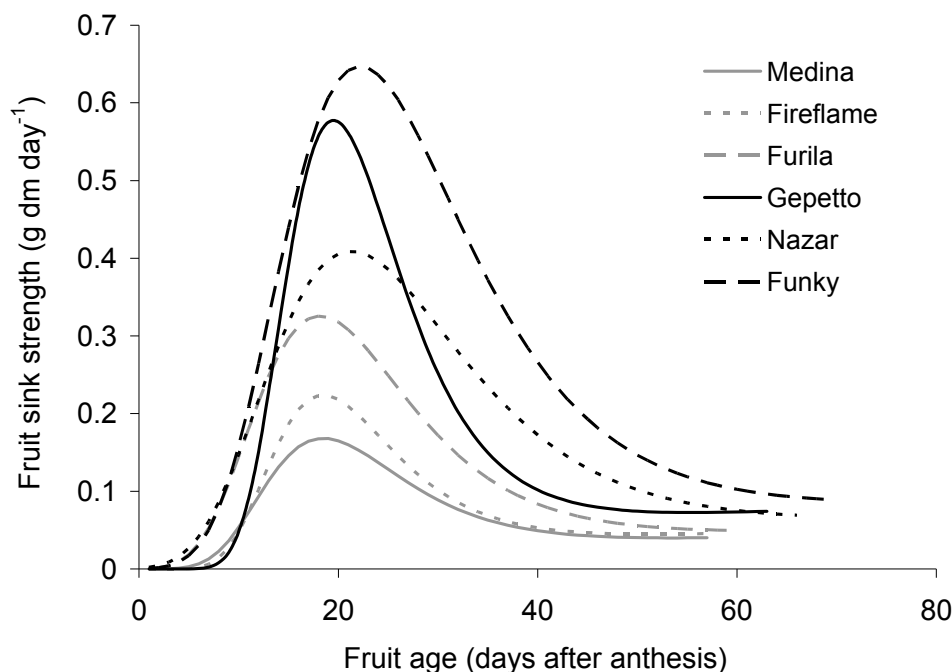


Fig. 5.1 Fruit sink strength curves for six different sweet pepper cultivars. Curves end at the average growth duration (time from anthesis until harvest ripe) of each cultivar.

Total fruit sink strength per plant differed between the cultivars (Fig. 5.3); all cultivars had an increasing total fruit sink strength in time, but strikingly the cultivars with the highest total fruit sink strengths were the cultivars with the highest fruit set ('Medina', 'Fireflame' and 'Furila', Fig. 5.3a-c). The total fruit sink strength of the large-fruited cultivars 'Gepetto', 'Nazar' and 'Funky' showed a wave-like pattern in time (Fig. 5.3d-f). For the latter two cultivars, the fruit set was maximal where the fruit sink strength was minimal and vice versa (Fig. 5.3e,f). The vegetative sink strength differed between the cultivars and was in general lower for the large-fruited cultivars than for the small-fruited cultivars (Table 5.2).

Table 5.2 Fruit growth duration, maximum potential fruit growth rate, ratio of actual to potential fruit weight, estimated vegetative sink strength and source-sink ratio at fruit set for each of the six cultivars. Standard error is given for the measured variables.

	Fruit growth duration (d)	Maximum fruit growth rate (g dm d ⁻¹)	Vegetative sink strength (g dm d ⁻¹)	Ratio actual/potential fruit weight (-)	Source-sink ratio at fruit set (-)
'Medina'	57 ± 0.19	0.18	1.9	0.52 ± 0.01	0.55 ± 0.004
'Fireflame'	58 ± 0.19	0.22	2.2	0.49 ± 0.01	0.53 ± 0.004
'Furila'	59 ± 0.23	0.35	2.9	0.45 ± 0.01	0.46 ± 0.004
'Gepetto'	63 ± 0.51	0.58	2.7	0.70 ± 0.02	0.74 ± 0.013
'Nazar'	66 ± 0.39	0.41	1.5	0.72 ± 0.02	1.06 ± 0.025
'Funky'	69 ± 0.49	0.65	1.6	0.73 ± 0.03	1.03 ± 0.026

The wave-like pattern in source-sink ratio was less clear than in total fruit sink strength due to day-to-day variations in source strength (Fig. 5.4). Nevertheless, three periods with high source-sink ratios are visible for the large-fruited cultivars ‘Nazar’ and ‘Funky’: weeks 15-17, 21-24 and 28-31 (Fig. 5.4b). The source-sink ratio was higher for these cultivars than for the small-fruited cultivars. This was related to the ratio of actual to potential fruit weight, which was higher for the large-fruited cultivars (Table 5.2), implying a higher supply of assimilates. The source-sink ratio at fruit set showed a distinctive division into two groups; fruits of small-fruited cultivars set at lower source-sink ratios than the fruits of large-fruited cultivars (Table 5.2).

Simulations

A higher potential fruit weight resulted in a lower simulated percentage fruit set and a larger variation in fruit set (Table 5.3). At the same time, average fruit weight increased but the ratio of actual to potential fruit weight was lower. The same happened with fruit set when the source-sink threshold for fruit set was increased, but fruits became heavier, indicating higher source-sink ratios. Different durations in fruit growth hardly affected the simulation results. If the flower appearance rate was decreased, the number of fruits set and the variation in fruit set decreased as well, but the percentage fruit set and the fruit weight increased. Increasing the source strength or decreasing the vegetative sink strength both increased the percentage fruit set and decreased the variation in fruit set, and the fruits became larger as well (Table 5.3). None of the changes in parameter values did at the same time decreased fruit set, increase variation of fruit set and result in relatively larger fruits. This means that more than one parameter was responsible for the differences in fruit set patterns.

In the experiment, the parameters were correlated, e.g. a higher potential fruit size was combined with a higher threshold for fruit set. When the simulations were done with parameter combinations observed in the experiment (Table 5.1), fruit set percentage decreased while variation in fruit set increased when parameters representing small-fruited cultivars were replaced by parameters for large-fruited cultivars (Table 5.3). At the same time, actual fruit weight and the ratio of actual to potential fruit weight increased. The summarised results of the simulations (e.g., % fruit set, C.V. for fruit set) were close to the experimental results for ‘Medina’ and ‘Funky’, although variation in fruit set was overestimated.

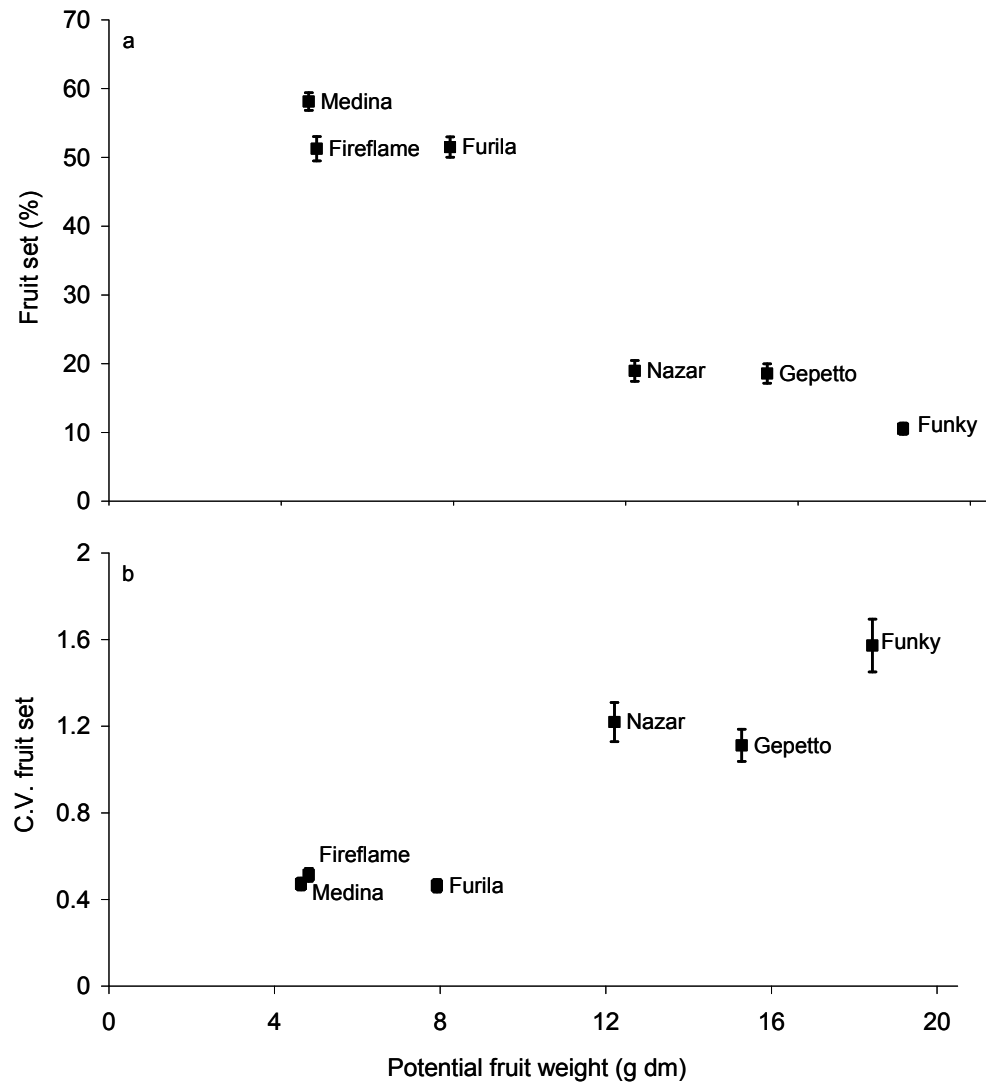


Fig. 5.2 Relationship between a) fruit set (%) and potential fruit weight (g dm), Spearman $R = -0.93$, $P < 0.001$, and b) variations in weekly fruit set and potential fruit weight (g dm), Spearman $R = 0.80$, $P < 0.001$, of six cultivars. Variations in weekly fruit set were defined as the coefficient of variation (C.V.), calculated as the standard deviation of weekly fruit set divided by average weekly fruit set. Error bars represent standard errors ($n = 12$) and are shown when larger than the symbol.

Table 5.3 Output of the simulation model for default simulation, simulations in which the parameters values were changed one-by-one and simulations with parameter values representing small-, medium- and large-fruited cultivars. Default simulation had 8 g dm as maximum fruit size, 0.5 as threshold for fruit set, fruit growth duration of 60 d, flower appearance rate of 0.5 d⁻¹, source strength of 2.4 g dm d⁻¹ and vegetative sink strength of 1.9 g dm d⁻¹.

Simulation	Changed parameter	Parameter value	Fruit set (#)	Fruit set (%)	Variation in fruit set (C.V.)‡	Fruit weight (g)*	Ratio actual/potential fruit weight (-)
Default			71	71	0.65	4.21	0.53
Changing parameters one-by-one	Potential fruit weight	4	100	100	0.15	2.51	0.63
		18	41	41	0.99	7.88	0.44
	Source-sink threshold for fruit set	1	26	26	1.46	6.45	0.81
	Fruit growth duration (d)	57	71	71	0.57	4.19	0.52
		70	70	70	0.64	4.19	0.52
	Flower appearance rate	1	88	44	1.00	3.59	0.45
	Source strength	2.2	64	64	0.69	4.09	0.51
		2.6	79	79	0.50	4.26	0.53
	Vegetative sink strength	1.6	76	76	0.55	4.30	0.54
		2.9	52	52	0.89	3.83	0.48
Small-fruited cultivar			123	62	0.69	2.03	0.51
Large-fruited cultivar			21	21	1.84	12.20	0.68

‡ C.V. is the coefficient of variation, representing the variation in weekly fruit set calculated as the standard deviation of the weekly fruit set divided by the average weekly fruit set

* of the harvested fruits

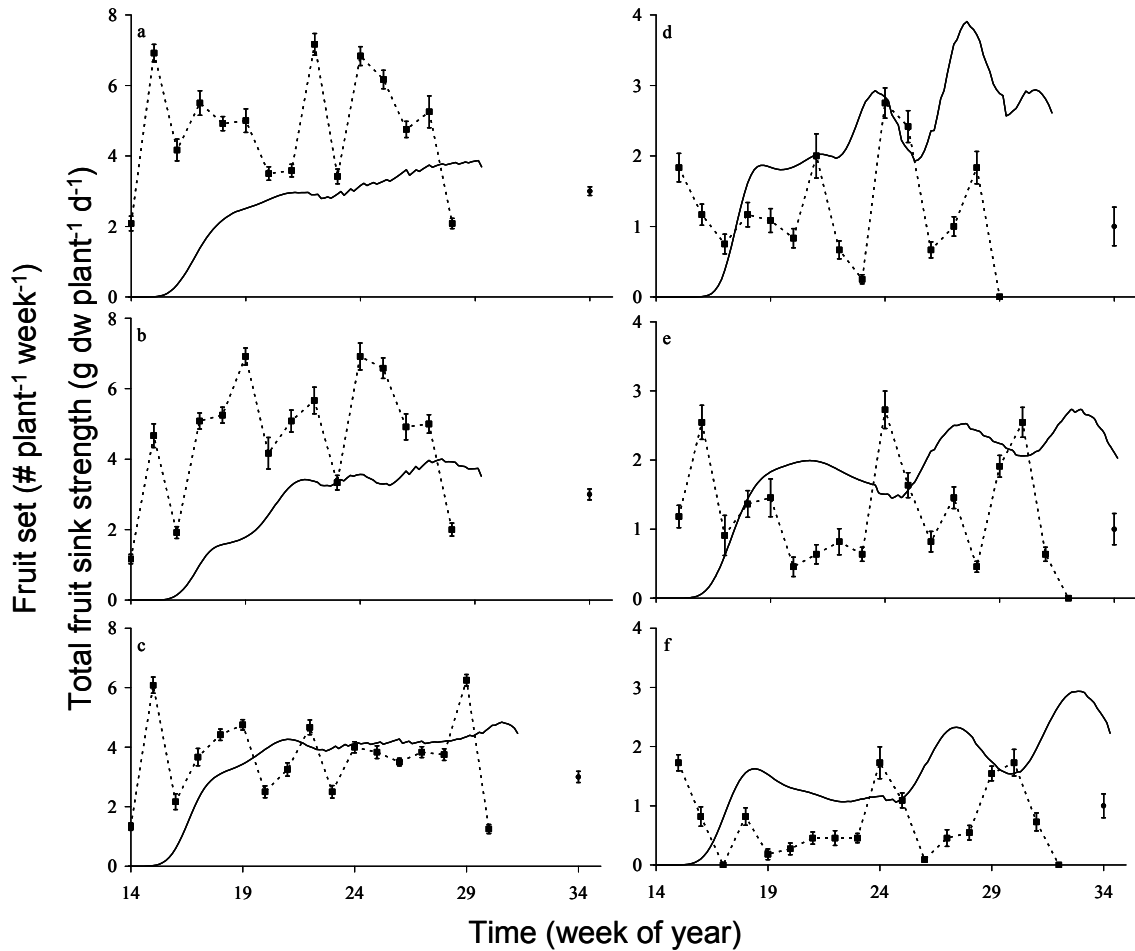


Fig. 5.3 Time course of total fruit sink strength (solid line) and weekly fruit set (symbols and dotted line) for ‘Medina’ (a), ‘Fireflame’ (b), ‘Furila’ (c), ‘Gepetto’ (d), ‘Nazar’ (e) and ‘Funky’ (f). Error bars at fruit set data points represent standard errors ($n = 12$), and error bar on the right-hand side represents average standard error of the sink strength.

Discussion

Several crops show cyclical patterns in fruit set and abortion (Schapendonk and Brouwer, 1984; Passam and Khah, 1992; Heuvelink *et al.*, 2004). In this paper we analysed the causes for these fluctuations, with *Capsicum* as a model plant. Marcelis *et al.* (2004) concluded that most of the variation in abortion of sweet pepper flowers/fruits can be related to the source and sink strength of the plant, which is confirmed by our data for the large-fruited cultivars. Fruit set in two of the three large-fruited cultivars was negatively correlated to total fruit sink strength (Fig. 5.3e,f). In these cultivars, fluctuations in fruit set during the growing season were in anti-phase with fluctuations in plant sink strength. In the other large-fruited cultivar (‘Gepetto’, Fig. 5.3d), fluctuations in total fruit sink strength and fruit set, averaged over twelve plants, were not exactly in anti-phase, due to high interplant variation in the timing of fruit set. Individual plants of this cultivar clearly showed this anti-phase. Cultivars with smaller-sized fruits showed relatively small fluctuations in fruit set and total fruit sink strength. In these cultivars, fruit set also occurred at high values of total

fruit sink strength. Hence, the results could not be explained by differences between the cultivars in fruit sink strength alone.

Besides individual fruit sink strength, the source-sink threshold for fruit set played a role in explaining differences in fruit set patterns of cultivars. The differences in source-sink threshold for fruit set can be interpreted as differences in sensitivity to fruit abortion. Cultivars are known to differ in their sensitivity to abortion, which is often related to high temperature stress (Aloni *et al.*, 1994; Sato *et al.*, 2004; Ledesma *et al.*, 2008) or low light availability (Turner and Wien, 1994a; Aloni *et al.*, 1996; Ferree *et al.*, 2001). The temperatures were not so high to cause stress (maximum daily temperature 27°C) and as the experiment was conducted in spring and summer, light levels were high. Temperature and light stress were therefore not likely in our experiment. Other factors, which have been related to differences in fruit set, were different numbers of seeds (Marcelis and Baan Hofman-Eijer, 1997), differences in sugar and starch content (Lebon *et al.*, 2004) or ovule development stage (Albuquerque *et al.*, 2002). These factors have not been investigated here, but might (partly) explain the different thresholds for fruit set. From an evolutionary point of view, it seems plausible that large-fruited cultivars will need a higher source-sink ratio for fruit set. Their fruits demand more assimilates. The higher threshold will reduce the chance that the plant starts investing in reproduction (fruits with seeds) which can not be successfully completed (too low source-sink ratio during fruit development). The measured levels of source strength, vegetative sink strength and flower appearance rate in large-fruited cultivars were favourable for fruit set (Table 5.3). Otherwise, fluctuations in these cultivars would have been even stronger.

Source-sink ratio is often used in simulation models to simulate fruit set (e.g., Lieth *et al.*, 1986; Bertin and Gary, 1993). Lieth *et al.* (1986) simulated the probability of abortion as a function of the source-sink ratio. Bertin and Gary (1993) used a threshold of source-sink ratio, below which fruit abortion increased linearly with decreasing source-sink ratio. According to these models, our assumption that fruit set occurs above a certain threshold source-sink ratio is an oversimplification. However, the average source-sink ratio at fruit set is a clear parameter to summarise differences between cultivars.

In a theoretical simulation study, Mathieu *et al.* (2008) showed that alternating patterns in organogenesis (e.g. fruit set) appeared when the demand for assimilates (sink strength) increased too much, causing a decrease in source-sink ratio. They also used a source-sink threshold to determine whether a fruit could be formed or not. When the threshold for fruit appearance was increased, fewer fruits appeared and the time spans between fruit set flushes increased, as the source-sink ratio was less often above the threshold value and for shorter periods of time. Our experiment demonstrated that also in real-life, the threshold for fruit set can be different between cultivars. However, the threshold was correlated to potential fruit size.

We conclude that fluctuations in fruit set are not only caused by differences in fruit sink strength, as stated in our hypothesis, but also by differences in the source-sink threshold for fruit set. Source strength only varied slightly between the cultivars in the current study and therefore, did not contribute to the differences observed in the fruit set patterns. However, source strength has a role in determining fruit set (Marcelis *et al.*, 2004). In other experiments, it might explain difference in fruit set patterns. The present research adds a new aspect, the source-sink threshold for fruit set, to the already existing knowledge on fruit set in relation to source and sink strength.

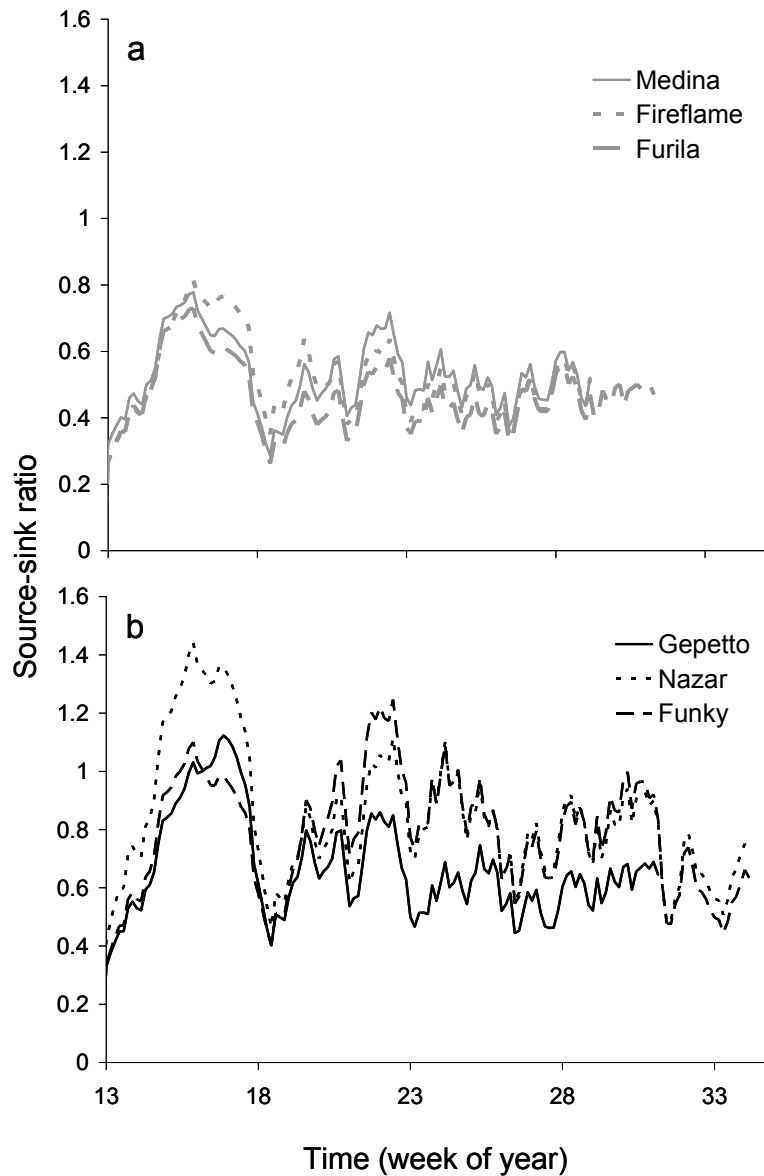


Fig. 5.4 Time course of source-sink ratio per plant for the six cultivars ($n = 12$). Lines are moving averages over five days. a) small-fruited cultivars, b) large-fruited cultivars.

The threshold for fruit set should be taken into account, especially when comparing fruit set in different cultivars. The results found in the current experiment are also likely to explain differences in fruit set between cultivars with different fruit sizes in other crops such as pumpkin (Stapleton *et al.*, 2000), melon (Valantin-Morison *et al.*, 2006) and cucumber (Jasso-Chaverria *et al.*, 2005). Physiological processes underlying the difference in source-sink threshold for fruit set should be the subject of further research.

Appendix Chapter 5

Equations used in the simple simulation model for fruit set

In the simple model used in Chapter 5, only fruit set is simulated using source and sink strength. The formulas used in the model are given below. State and rate variables are given in Table A5.1, parameters and their default values in Table A5.2, together with their description.

The age of fruit i , t_{f_i} , is expressed in days. Sink strength of fruit i of age t_{f_i} on day t_c (with t_c representing the day number in the simulation), $Sink_{f_i(t_{f_i})}(t_c)$, as quantified by the potential growth rate, is calculated by the derivative of the Gompertz function.

$$Sink_{f_i(t_{f_i})}(t_c) = w_{\max} \cdot k \cdot e^{-e^{-k(t_{f_i}-t_m)}} \cdot e^{-k(t_{f_i}-t_m)}$$

Fruit growth duration, the time between anthesis and harvest, is a parameter in the model, called FGD.

This sink strength for fruit i is calculated daily between anthesis and harvest. A fruit is removed from the simulation when its age t_{f_i} equals the age of harvest (FGD).

On an arbitrary simulation day, fruits of different ages and hence fruits with different sink strengths are present. The total fruit sink strength on day t_c , $Sink_{TF}(t_c)$ is calculated from the sink strength of all n_{t_c} fruits present on that day.

$$Sink_{TF}(t_c) = \sum_{i=1}^{n(t_c)} Sink_{f_i(t_{f_i})}(t_c)$$

The vegetative sink strength, $Sink_v$, was assumed constant over time. From the total fruit sink strength on day t_c and the vegetative sink strength, the total sink strength on day t_c ,

$Sink_{TOT}(t_c)$, is calculated as:

$$Sink_{TOT}(t_c) = Sink_v + Sink_{TF}(t_c)$$

The source strength, $Source$, is the daily growth rate of the plant and is constant over time. From the total sink strength on day t_c and the source strength the source-sink ratio on day t_c ,

$SSR(t_c)$, is calculated as:

$$SSR(t_c) = \frac{Source}{Sink_{TOT}(t_c)}$$

The appearance of new flowers is regulated by the parameter FLOWER. Per day, at most one flower can appear. On a day that a flower appears, the source-sink ratio of that day,

$SSR(t_c)$, is compared to the threshold for fruit set, $THRES_{FS}$.

If $SSR(t_c) > THRES_{FS}$, the fruit will set and continues to grow till harvest, else the fruit aborts and is removed from the simulation.

Each day, all these variables are calculated. After these calculations, the day number t_c and the fruit ages t_{f_i} are increased by 1 and all the steps are repeated.

Table A5.1 State and rate variables of the model with their units.

Variable	Unit*	Explanation
t_c	d	Simulation day (1=start simulation)
t_{f_i}	d	Age of fruit i
$Sink_{f_i(t_{f_i})}(t_c)$	g dm fruit ⁻¹ d ⁻¹	Sink strength of fruit i with age t_{f_i} on day t_c
$Sink_{TF}(t_c)$	g dm plant ⁻¹ d ⁻¹	Total sink strength of all fruits of a plant on day t_c
$Sink_{TOT}(t_c)$	g dm plant ⁻¹ d ⁻¹	Total sink strength (fruits and vegetative parts) on day t_c
$SSR(t_c)$	-	Source-sink ratio on day t_c

* dm = dry mass

Table A5.2 Parameters of the model with their default values and units.

Parameter	Default value	unit	Explanation
w_{max}	8	g dm	Potential fruit size
k	0.095	d ⁻¹	Rate parameter of Gompertz function
t_m	20	d	Time of maximum growth
FGD	60	d	Fruit growth duration
$Sink_v$	1.9	g dm plant ⁻¹ d ⁻¹	Vegetative sink strength
Source	2.4	g dm plant ⁻¹ d ⁻¹	Source strength
FLOWER	0.5	d ⁻¹	Flower appearance rate
$THRES_{FS}$	0.5	-	Source-sink ratio above which a fruit sets

Chapter 6

Survival analysis as a tool to quantify effects of factors on abortion rates of reproductive organs

Abstract

*Survival analysis is a method to analyse timing of stochastic events and to quantify the effects of factors thereon. Abortion of reproductive organs is a highly variable process mainly depending on source and sink strength. We hypothesise that the effect of source and sink strength on abortion rate can be explained with a stochastic model, obtained with survival analysis. Flower and fruit abortion in *Capsicum annuum* as observed in a temperature and a planting density experiment were analysed. Source and sink strength were used as explanatory variables. Increasing source strength as well as decreasing sink strength decreased the abortion rate. The effect was non-linear, source strengths above 6 g CH₂O plant⁻¹ d⁻¹ did not decrease abortion rates further. Fruit on side shoots had 32 to 57% higher abortion rates than fruit on the main shoot. The maximum abortion rate occurred around 100°Cd after anthesis. The survival analysis is shown to be a powerful technique to analyse and quantify abortion rates and the effects of source and sink strength as time-dependent covariates. We discuss the advantages and disadvantages of the use of survival analyses for these kind of data.*

Introduction

In various research fields, the interest arises whether a certain event occurs, and how long it takes before this event occurs, depending on certain factors. The time-to-event is not always known for all individuals and the time-to-event data are usually not normally distributed. Time-to-event data can be analysed with survival analysis (Kleinbaum and Klein, 2005). The time until the occurrence of an event can be related to explanatory variables, the so-called covariates. The use of survival analysis in plant and crop science is scarce, although increasing (e.g. Ojiambo and Scherm, 2005; Winkler *et al.*, 2005; Esker *et al.*, 2006). Survival analysis is often taken as an alternative for, or as complementary to, describing processes with growth equations (Fox, 1993; Dungan *et al.*, 2003; Vange *et al.*, 2004). Dungan *et al.* (2003) suggest that survival analysis is useful in cases when there is one single transition (e.g. from alive to dead) and when the division of a population in cohorts, used in traditional demographic approaches, is difficult. Up to now most examples in plant and crop science are limited to univariate analyses with fixed covariates, while more complex analyses with more explanatory power are possible.

Flower and fruit abortion is a yield-limiting factor in many crops (Goldschmidt, 1999; Halbrech *et al.*, 2005; Bacci *et al.*, 2006). Abortion of these organs can be caused by unfavorable conditions such as temperature stress (Guilioni *et al.*, 1997), low light conditions (Ferree *et al.*, 2001), and competition from fast growing fruit (Marcelis *et al.*, 2004). Factors affecting abortion are often summarized in the terms source and sink strength, which represent the supply and demand of assimilates, respectively. The qualitative relations between flower and fruit abortion and the influencing factors are often clear, e.g. more flowers abort when more competing fruit are present, or when radiation levels are lower. However, quantifying the effect of these factors on fruit abortion is difficult, but is necessary for predicting crop response to environmental factors and the subsequent yield.

When frequent observations on dates of anthesis, flower and fruit abortion and fruit harvest are done, survival times of flowers and fruit can be calculated. Not all fruit abort: some are harvested and other fruit are still growing by the end of the experiment. These observations are censored. Survival analysis can take these censored observations into account, and is therefore the appropriate tool to estimate the rate at which abortion occurs. When additional measurements on plant growth, development, and environmental circumstances are obtained, the effect of these factors on abortion can be quantified.

We applied survival analysis on two data sets of sweet pepper containing the above described observations. Factors known to influence flower and fruit abortion, summarised in source and sink strength, were used as covariates. An alternative for sink strength, which is not only based on assimilate demand, but also takes hormonal dominance into account (Bangerth, 1989), was applied as well. We hypothesise that the effect of source and sink strength on abortion rate can be explained with a stochastic model, obtained with survival analysis. Questions to be answered are whether the application of survival analysis gives results which are in line with the physiological knowledge, and if survival analysis could give additional information which is otherwise not acquired from the experiments. We discuss the advantages and disadvantages of the use of survival analyses for these kind of data.

Materials and methods

Survival analysis

Survival analysis comprises techniques to analyse time-to-event data. Also the survival times of individuals for which the studied event did not yet happen can be used in the analysis (the so-called censored observations). With the Kaplan-Meijer product limit estimator (Kleinbaum and Klein, 2005), empirical survival curves are estimated. Differences between treatments can be checked visually and can be tested for significance. The effects of covariates on the abortion rate can not be quantified with this technique. A popular method for quantifying the effect of covariates is the Cox proportional hazards model (Kleinbaum and Klein, 2005). Fixed covariates are factors with a constant value during the survival time of the individual. The method can also deal with time-dependent covariates, from which the value can vary over time, for instance, from day to day. With time-dependent covariates, the model is called the extended Cox model. The Cox model works with a hazard rate, i.e. the probability of occurrence of the event per unit time, given the event has not yet occurred (equation 6.1).

$$h_{f(t_f)}(t_c, X) = h_{0,f(t_f)}(t_c) * \exp(\omega_j) * \exp\left[\sum_{i=1}^n \beta_i X_{ij}(t_c)\right] \quad \text{equation 6.1}$$

In our case, $h_{f(t_f)}(t_c, X)$ is the abortion rate (the hazard rate) at calendar time t_c of fruit f with age t_f on plant j , influenced by n fixed or time-dependent covariates $X_{ij}(t_c)$ ($i=1,...,n$; $j=1,...,m$): $h_{0,f(t_f)}$ is the baseline abortion rate of a fruit with age t_f , which is multiplied by two exponential expressions. The first exponential expression is the so-called frailty term, which is equivalent to a random effect in a mixed model. It allows for grouping of individuals, here flowers and fruit on the same plant. The frailty ω_j has a specified distribution (gamma, Gaussian, or Students' t) with mean zero and unknown variance θ (Therneau, 2008). The second exponential expression takes the effect of the covariates on the abortion rate into account. Here, the values of the covariates at calendar time t_c are denoted by $X_{ij}(t_c)$ and β_i is the coefficient quantifying the effect of the covariate X_{ij} on the abortion rate. If X_{ij} is a fixed covariate, $X_{ij}(t_c)$ is constant. A negative coefficient β_i of covariate i decreases the baseline abortion rate, and a positive coefficient β_i of covariate i increases the baseline abortion rate.

The Cox model is semi-parametric; the baseline hazard $h_{0,f(t_f)}$ has no pre-defined shape. It is estimated simultaneously with the regression coefficients β_i ($i = 1,...,n$) for the covariates and the frailty ω_j ($j=1,...,m$). The estimation is done by maximising the partial likelihood function based on the data (Kalbfleisch and Prentice, 2002).

The Cox model assumes a linear relationship between the covariate and its effect on the log hazard. With splines it can be investigated whether the linear form is the best functional form or whether the response of the log hazard to the covariate is non-linear (Therneau and Grambsch, 2000).

Description of experiments

Data of two experiments were used to quantify abortion rates of sweet pepper flowers and fruit and the factors affecting abortion: a temperature experiment and a planting density experiment. In both experiments, the two first-order branches of each plant were retained.

Of each next dichotomous branching, the weaker branch was pruned just above the first leaf (= side branch), while the stronger one was allowed to grow (= main branch).

Temperature experiment: Sweet pepper plants *Capsicum annuum* cv. 'Mazurka' (Rijk Zwaan, De Lier, the Netherlands) were grown in three air-conditioned compartments (2.5 m by 5.4 m) of a Venlo-type glasshouse in Wageningen (The Netherlands, 52°N) from March to half July 2007. Average 24h temperatures were set at 18°C, 21°C and 24°C, with four degrees difference between day and night. Day was between 8am and 6pm, and night between 8pm and 6am, and the hours in-between were used for heating up and cooling down, respectively. The realised mean temperatures were $18.3^{\circ}\text{C} \pm 0.5$, $20.3^{\circ}\text{C} \pm 0.35$ and $23.3^{\circ}\text{C} \pm 0.31$ (mean \pm sd). The average daily global outside radiation was 15.5 ± 2.9 MJ $\text{m}^{-2} \text{d}^{-1}$ (mean \pm sd). Transmission of the glasshouse was 30%. The vapour pressure deficit of the air was kept constant at 0.7 kPa, implying an average relative humidity of 67%, 73% and 77%, for 18°C, 21°C and 24°C respectively. CO₂ concentration was 455 $\mu\text{mol mol}^{-1}$. Plants were grown in 15 l pots, filled with commercial potting medium. Planting density was 4.7 plants m^{-2} . Water was given daily, and fertilization with a standard nutrient solution (PG-mix, 12+14+24+2) was done once or twice a week, depending on plant growth.

Density experiment: Sweet pepper plants *Capsicum annuum* 'Red Spirit' (Enza seeds, Enkhuizen, the Netherlands) were grown in a Venlo-type glasshouse in Wageningen from December 2001 to early September 2002 at planting densities of 2.5, 3.8, and 5 plants m^{-2} . The treatments were arranged in a randomized complete block design consisting of 7 blocks with 3 plots per block. The realised average daily temperature during the experiment was $21.6^{\circ}\text{C} \pm 2.0$ (mean \pm sd). The average global outside radiation was 11.6 ± 7.7 MJ $\text{m}^{-2} \text{d}^{-1}$ (mean \pm sd). Transmission of the greenhouse was 65%. The average CO₂ concentration during the day was 430 $\mu\text{mol mol}^{-1}$ and the average daily relative air humidity was about 75%. Plants were growing in rockwool slabs. Nutrient solution was prepared according to Voogt and Bloemhard (1993) and was supplied by trickle irrigation.

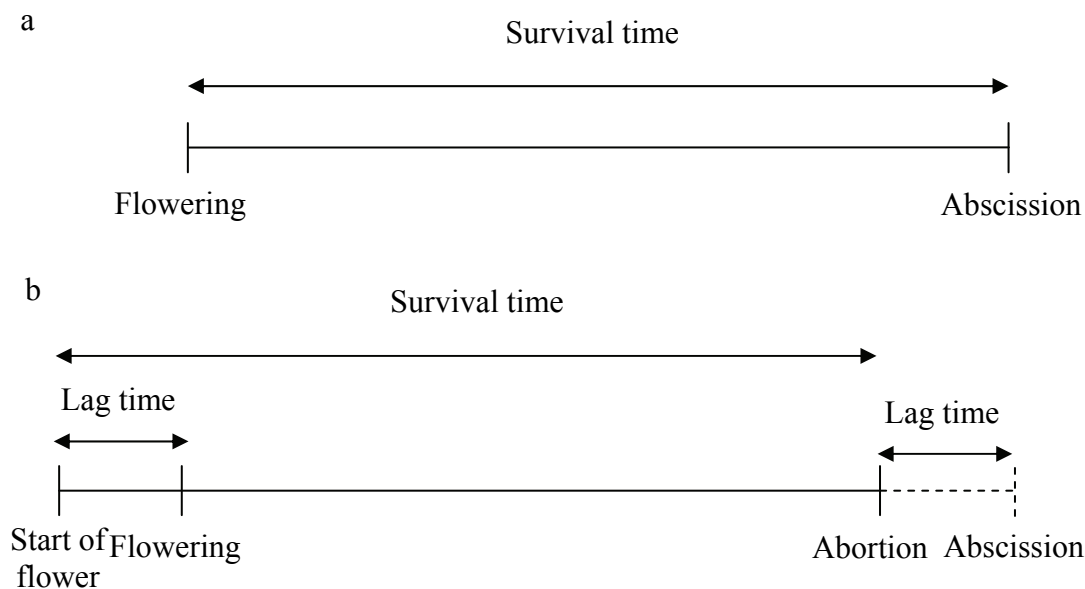


Fig. 6.1 Visualisation of the time shift in the start and end points of the survival times of aborted flowers and fruit when a lag time is applied. a) original observations for an aborted flower or fruit, b) survival time shifted by lag time for an aborted flower or fruit.

Observations and measurements

In each experiment, anthesis dates were recorded, as well as flower and fruit abscission dates and fruit harvest dates. Eight plants per treatment were observed in the temperature experiment and seven plants per treatment (one per block) in the density experiment. Frequency of the observations was six times per week in the temperature experiment and two times per week in the density experiment. Abscission is defined as the drop of a flower or fruit. Note that in the following the terms abortion or abscission refer to abortion or abscission of both flowers and fruit. In total, survival times of 172, 370 and 515 flowers were collected at 18°C, 21°C and 24°C, respectively, and survival times of 843, 851 and 826 flowers at 2.5, 3.8 and 5 plants m⁻², respectively.

Five and seven destructive harvests were conducted in the temperature and density experiment, respectively. Six to nine plants per treatment were destructively harvested. From these plants, leaf area and dry weight of leaves, stems and fruit were registered. Leaf area was measured using an area meter (LI-COR, model 3100, Lincoln, NE, USA). Dry weights of stems and leaves were determined after drying for one night in an oven at 105°C. Fruit were dried during two nights. Completely red fruit were harvested twice a week and their fresh and dry weight were measured.

Calculating survival time

The survival time derived from the observations started at anthesis and ended when abscission of the flower or fruit was observed, when the fruit was harvested, or when the experiment ended. The latter two were censored survival times. Survival times were expressed in degree days, calculated with a base temperature of 10°C (Marcelis *et al.*, 2006).

The observed event was abscission of the flower or fruit, however, the event of interest is abortion. Abscission takes place after the abortion process has been completed. A lag time was therefore assumed between abortion and abscission. However, if the lag time would be subtracted from the survival time, some of the aborted flowers should be omitted from the analysis, as their survival time would become negative. The survival time of the aborted flowers and fruit was therefore kept as observed, i.e. time between anthesis and abscission, but the starting point of the survival time was shifted to an earlier time, namely the number of degree-days of the considered lag time before anthesis. The estimated abortion time was the considered lag time (in degree-days) before the observed abscission time (Fig. 6.1a,b). The shift in starting time was applied to all fruit. As a consequence, the survival time of harvested and otherwise censored fruit was extended by the lag time. A lag time of 50°Cd was used, which in preliminary analyses had higher likelihood than lag times of 30°Cd or 70°Cd. This was in line with results from Aloni *et al.* (1991), who found that sweet pepper flowers abscised 3 to 5 days after application of heat stress.

Calculating source and sink strength

For both experiments, the source strength during each day of the whole cultivation period was obtained with model simulations. Leaf area data from the destructive measurements, and recorded radiation, temperature and CO₂ concentrations were used to simulate growth of the sweet pepper plants with the INTKAM model, adapted for sweet pepper (Marcelis *et al.*, 2006). Simulated total dry matter production was calibrated on the measured total dry matter production of all destructive harvests. Source strength was defined as the daily gross assimilation minus maintenance respiration and expressed in CH₂O plant⁻¹ d⁻¹.

Total plant sink strength was calculated for the plants on which observations of anthesis, fruit abortion and fruit harvest were performed. It was calculated as the sum of the total generative sink strength and the vegetative sink strength. The total generative sink strength of a plant was calculated for each day as the sum of the sink strengths of the individual fruit. Application of the lag time influenced the total generative sink strength: the sink strength of a flower or fruit was not calculated between abortion and abscission. The sink strength was zero before anthesis. The sink strength was quantified as the potential fruit growth rate, derived from additional measurements in the temperature experiment. Fruit growth was obtained as described in Chapter 3, using a Richards function to describe fresh weight growth. To obtain the potential fruit growth, the asymptote of the Richards function was increased to represent the average fruit weight of the 10% largest fruit. The sink strength of a fruit was expressed in $\text{g CH}_2\text{O d}^{-1}$.

The increase in vegetative sink strength with increasing temperature was estimated from the temperature experiment. Per temperature treatment, the vegetative sink strength was estimated by manual iteration using simulated source strength, calculated total fruit sink strength and observed dry matter partitioning into the fruit at the destructive harvests. The total squared deviation between measured and calculated dry matter partitioning into the fruit for the destructive harvests was minimised. A linear relationship between vegetative sink strength and measured temperature was fitted. The vegetative sink strength ($\text{g CH}_2\text{O plant}^{-1} \text{d}^{-1}$) was $-2.28 + 0.16T$, where T is the average 24h temperature ($^{\circ}\text{C}$). For the density experiment, the intercept of the relation between vegetative sink strength and temperature was re-estimated at -1.06 , but the slope was kept the same as in the temperature experiment.

Performed survival analyses and model selection

Kaplan-Meijer analysis; First, the survival times were analysed using the Kaplan-Meijer product limit estimator. Empirical survival curves were constructed for the three temperatures and for the three densities. Survival times started at anthesis and ended at abscission or the censored survival time. The difference in survival between the treatments was tested with the log-rank test.

Extended Cox model; Next, survival analyses with the extended Cox model were performed with time-dependent covariates source and sink strength. In these analyses, the lag time of 50°Cd between abortion and abscission was applied. Besides daily values of source and sink strength, averages over the past two to ten days were tried, as it was unlikely that if circumstances were unfavourable for only one day (e.g. low radiation), abortion of the fruit was induced. All combinations of averages of source and sink strength (100 in total) were tried. The best combination of source and sink strength was selected by Akaike's Information Criterion (AIC; equation 6.2).

$$\text{AIC} = -2 \ln(\text{likelihood}) + 2p \quad \text{equation 6.2}$$

The AIC takes into account the likelihood of the fitted model as well as the number of parameters p used in the model. Minimum AIC indicates the best model.

The Cox model assumes a linear relationship between the covariate and its effect on the log-hazard. The analysis was improved by assessing the functional form for the covariates. By fitting a spline function instead of only a linear term for the covariates, the

functional form of the covariates is found. With a polynomial function the form of the spline was approximated. Initially, each covariate was fitted with a fourth order polynomial, and non-significant terms were eliminated one by one.

Other covariates derived from source and sink strength have been used as well in the survival analysis, but these explained the survival times less well (Appendix I).

Alternative for fruit sink strength; At each day, the ages (in °Cd) of all flowers and fruit on the plant were known. The flowers and fruit were divided into age classes of 50°Cd width. The number of flowers and fruit present in each age class on each day was counted and used as time-dependent covariates. Univariate analyses were done in which only one age class was used as a covariate. The impact of each age class was compared within and between the experiments. Next, a multivariate analysis was done which included source strength, vegetative sink strength and all age classes. Subsequently, the least significant age class was removed and the difference between two models was tested with the likelihood ratio (LR) test. This test compares the log-likelihood ratio of the full and the reduced model: $2 * (\ln(\text{likelihood}_{\text{full}}) - \ln(\text{likelihood}_{\text{reduced}}))$. The LR-test has a chi-square distribution with degrees of freedom equal to the difference between the degrees of freedom of the full and the reduced model. If there was no difference between the reduced and the full model ($P > 0.05$), the age class was omitted. In the final model, a check for non-linearity between the covariates and the log hazard revealed that the assumed linear relationship was correct (data not shown).

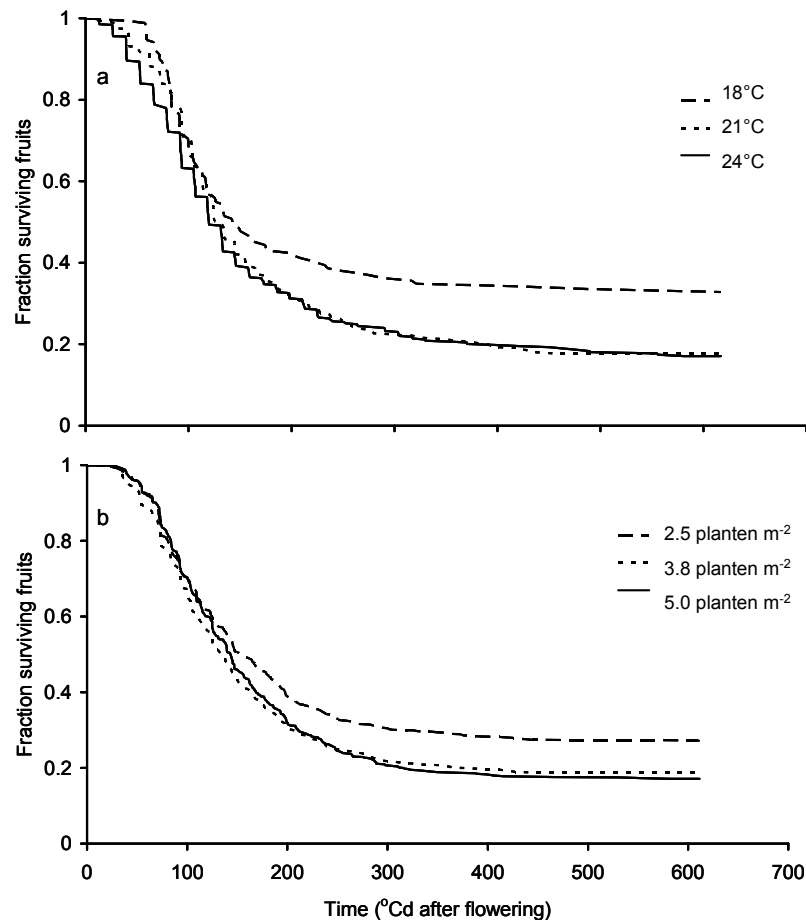


Fig. 6.2 Kaplan-Meijer survival curves of the three treatment levels in the temperature experiment (a) and the density experiment (b).

The probability of abortion of fruit on the same plant is more correlated than for fruit on different plants, due to unaccounted effects like shading or local differences in temperature. A random effect for plant, the frailty term, was therefore included in all analyses. The degrees of freedom associated with the frailty were not preset (Therneau and Grambsch, 2000). Both gamma and Gaussian distributions were tried for the variance of the frailty. There was only a very slight difference in AIC between the models with Gaussian frailty and gamma frailty (data not shown). The results of analyses with the Gaussian frailty distributions are shown.

Position of the flower or fruit, whether it was on the continuous main stem or on the pruned side stem, influences the abortion rate (Wubs *et al.*, 2007). This factor was added to the final model with source and sink strength and to the reduced model with age classes.

All analyses were done in the R language version 2.8.0 (R core development team, 2007).

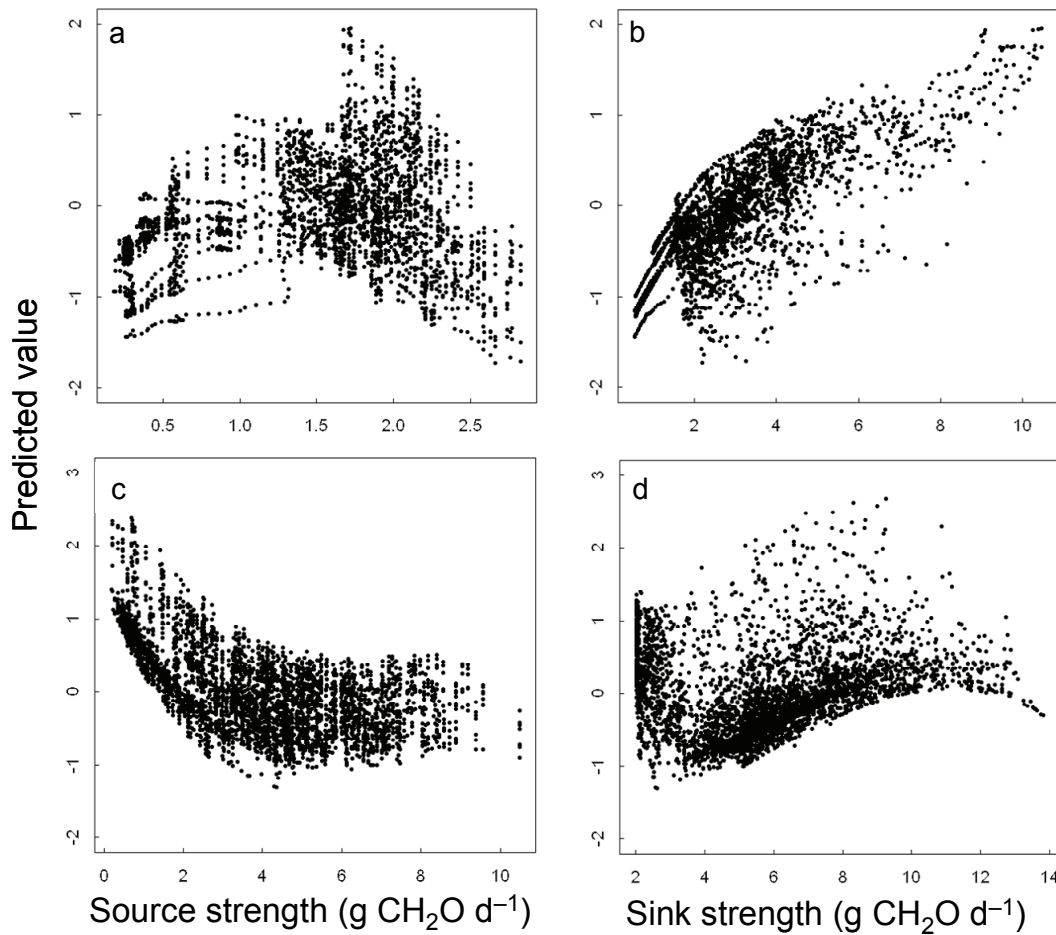


Fig. 6.3 Predicted values of the polynomial model with source and sink strength as covariates. a,b) temperature experiment, c,d) density experiment. The predicted value is the term $\sum_{i=1}^n \beta_i X_{ij}(t_c) + \omega_j$, where X_{1j} is the source strength of plant j and X_{2j} sink strength of plant j . All models include a frailty term ω_j , implying that the predicted values of flowers from different plants with the same source and sink strength is different. The predicted values are scaled to 0 at the average predicted value.

Goodness-of-fit

Goodness-of-fit of a model is often assessed by comparing observed and predicted values. However, constructing predicted survival curves from analyses with time-dependent covariates is not possible in this case, as future values of the covariate are often unknown and if these values are known, it implies that the individual is still at risk (Fisher and Lin, 1999). Also other methods commonly applied in regression analysis for assessing goodness-of-fit, like plotting residuals against fitted values to observe randomness of residuals, are not valid for Cox models (Therneau and Grambsch, 2000). A R^2 value can be calculated, but is often very low, even for a perfectly adequate model (Stevenson, 2009). Goodness-of-fit was assessed by calculating the change in parameter value when one data point was removed and by visual inspection of the martingale residuals.

Results

Kaplan-Meijer survival curves

Reproductive organs from plants growing at 18°C had a higher survival than reproductive organs on plants growing at 21 or 24°C ($P < 0.001$, Fig. 6.2a). Reproductive organs from plants growing at 2.5 plants m^{-2} showed significantly higher survival than reproductive organs on plants growing at 3.8 and 5.0 plants m^{-2} ($P < 0.001$, Fig. 6.2b). In both experiments, most flowers and young fruit aborted within 150°Cd after anthesis.

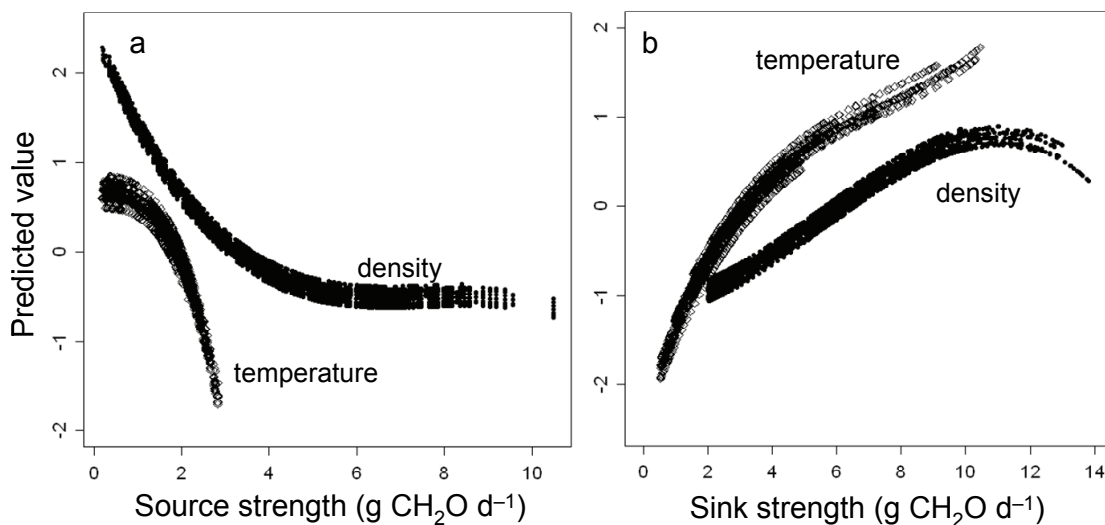


Fig. 6.4 Response of the predicted value on source and sink strength. The term $\beta_i X_{ij}(t_c)$ is only calculated for source strength or sink strength. The frailty term for plant, ω_j , is included in each data point, resulting in different predicted values at the same source or sink strength. The predicted values of each covariate in each experiment are scaled to 0 at the average predicted value of the covariate experiment combination. The sum of the predicted value for source strength and for sink strength of combination of source and sink strength is the predicted value at similar source and sink strength from Fig. 6.3.

Cox model

Covariates source and sink strength; The best combination of moving averages of source and sink strength in the temperature experiment was both source and sink strength averaged over 10 days (AIC 9816). For the density experiment, the best fit was obtained when source strength averaged over two days and sink strength averaged over 10 days (AIC 26546).

The analysis was improved when a spline function was used for source and sink strength; the use of the spline function decreased the AIC to 9721 and 26240 in the temperature and density experiment, respectively.

In the temperature experiment, the polynomial function approximating the spline contained a third order term for source strength and first, second and third order terms for sink strength (AIC 9794). The term $\sum \beta_i X_{ij}(t_c)$ from equation 6.1 quantifies the effect of the source and sink strength on the abortion rate. When this term is calculated for each combination of source and sink strength and the β_i 's from the polynomial function, the shape of the effect of source and sink strength on the abortion rate can be studied (Fig. 6.3). There was only a very slight decrease in the abortion rate when the source strength increased (Fig. 6.3a). The increase of the abortion rate with increase in sink strength was much stronger (Fig. 6.3b). When the term $\beta_i X_{ij}(t_c)$ was calculated only for source strength or sink strength (Fig. 6.4a,b), the effect of source strength appeared to be strong as well, especially between 1.5 and 2.5 g CH₂O d⁻¹, but the effect of sink strength was even stronger.

In the density experiment, the polynomial function which approximated the spline had first, second and third order terms for source strength and second and third order terms for sink strength (AIC 26333). For source strength values lower than 6 g CH₂O plant⁻¹ d⁻¹, there was strong negative effect on the hazard with increase in source strength (Fig. 6.3c). The effect on the hazard at low sink strengths (<3 g CH₂O plant⁻¹ d⁻¹) varied largely (Fig. 6.3d), due to a wide range of source values combined with low sink values. The sink strength increased the hazard rate when values were above 3 g CH₂O plant⁻¹ d⁻¹. When the term $\beta_i X_{ij}(t)$ was calculated only for source strength or sink strength (Fig. 6.4a,b), it appears that the source strength higher than 6.0 g CH₂O d⁻¹ did not further decrease the hazard rate. Sink strength values higher than 10.0 g CH₂O d⁻¹ decreased the hazard rate (Fig. 6.4b). These sink values only occurred at source strengths higher than 5.0 g CH₂O d⁻¹, and accounted for the negative effect on the abortion rate expected from the source strength.

In both analyses, addition of the position of the fruit on the plant (whether it was on the main stem or on the side stem) improved the polynomial fit (AIC 9772 and 26288 for the temperature and density experiment, respectively). The coefficient for position was 0.355 and 0.316 in the temperature and density experiment, respectively. This implied that the abortion rate was 35% or 32% higher for a fruit on the side branch than for one on the main branch. Addition of this factor hardly affected the values of the other coefficients.

The cumulative baseline hazards of the polynomial model in the temperature and density experiment were similar in shape, but not in actual values (Fig. 6.5). The value of the hazard rate was lower in the temperature experiment, but the combination of source and sink strength mostly increased the baseline hazard in the temperature experiment (the term $\sum_{i=1} \beta_i X_{ij}(t_c)$ is higher than zero), while it was mostly decreased in the density experiment.

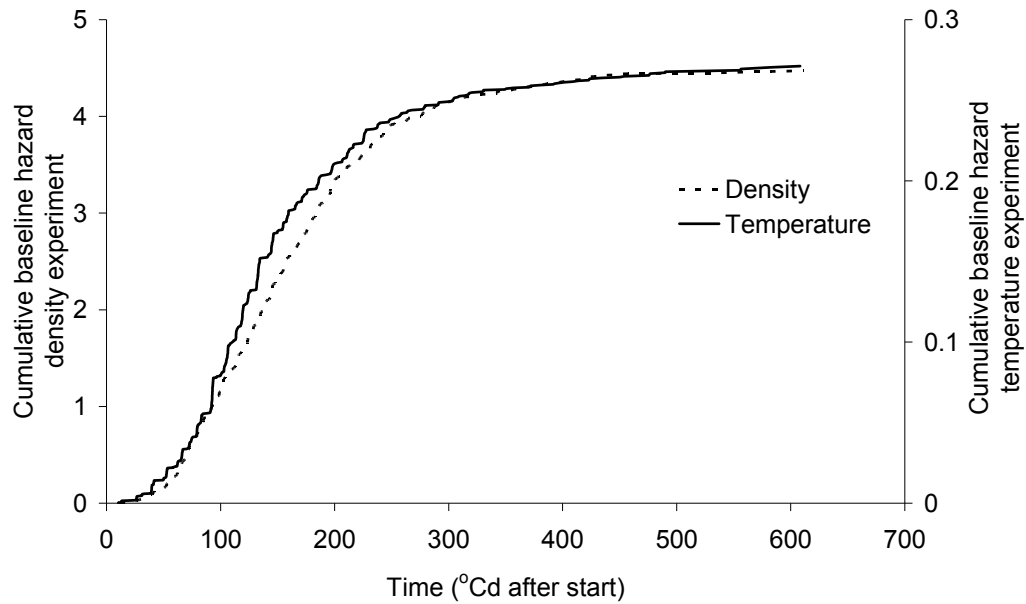


Fig. 6.5 Cumulative baseline hazards for the temperature and density experiment when the analysis was done with a polynomial function for source and total sink strength and the position of the flower/fruit on the plant. Lag time was 50°Cd in both analyses

Age classes as covariates; In the univariate analyses, the age classes which had most effect on abortion of flowers and fruit were not exactly the same in the two experiments (Fig. 6.6). The pattern was similar, but seemed to have shifted slightly to the right in the temperature experiment, which might be due to the use of different cultivars. The age classes affecting the probability of abortion most were also age classes with the higher sink strengths (age classes around 350°Cd, which is 300°Cd after flowering). However, also some older age classes (around 650°Cd) increased the abortion rate, which was unexpected based on physiological knowledge. Young age classes (0-200°Cd) and in the density experiment, age classes older than 700°Cd, decreased the abortion rate. The presence of young fruit indicates that the circumstances for fruit set are favourable, and hence the abortion rate is decreased. Older age classes indicate presence of nearly ripe fruit, which exhibit hardly any competition with other fruit, thus indicating favourable circumstances for fruit set as well. Hence, these age categories indicate indirect effects.

Next, analyses including source strength, vegetative sink strength and all age classes were conducted. In the density experiment, the vegetative sink strength was not significant and was omitted from the analysis. For the temperature experiment, the influence of each age class on the baseline hazard was comparable to Fig. 6.6a. For the density experiment, fruit aged 250-450°Cd and 800-850°Cd increased the abortion rate most. The coefficient for source strength was lower in the temperature experiment (−0.446) than in the density experiment (−0.314). The cumulative baseline hazard had a similar shape as for the analyses with source and total sink strength and was again higher for the density than for the temperature experiment (figure not shown). The analyses with indication of fruit in age classes had an equal (density experiment) or better (temperature experiment) fit than the models with polynomials of source and sink strength; the AIC was 9774 and 26333 for the temperature and density experiment, respectively. Adding the position of the flower/fruit on the plant to the analysis decreased the AIC (9738 and 26286 for the temperature and density experiment, respectively). The coefficient for position was 0.325 in the density experiment,

which is comparable to the value in the model with source and sink strength, but the coefficient was higher in the temperature experiment (0.454).

Goodness-of-fit

The influence of individual observations on the coefficient β was very small when polynomial functions of source and sink strength were used as covariates (Table 6.1). In the temperature experiment, the average absolute change in the coefficient β for the third order term of source strength when a data point was removed was 0.0000165. Compared to the value of β (−0.1046), this is only 0.016%. The third order term of the polynomial is often most sensible to removal of individual data points (Table 6.1). In the analysis with indication of fruit in age classes, the age class 700-750°Cd had in both data sets the highest absolute deviation.

Inspection of the martingale residuals revealed that in the temperature experiment the fit was poor at very high values of sink strength ($>8 \text{ g CH}_2\text{O d}^{-1}$) combined with intermediate values of source strength ($1.6\text{-}2.0 \text{ g CH}_2\text{O d}^{-1}$), which is 1.62% of the cases (data not shown). In the density experiment, combinations of low source strength ($<2 \text{ g CH}_2\text{O d}^{-1}$) and intermediate sink strength ($6\text{-}9 \text{ g CH}_2\text{O d}^{-1}$) the fitted poor in the density experiment. This was the case in about 0.7% of the data. In the analyses with age classes, high residuals were found at similar low source strengths as in the analysis with source and sink strength, which occurred in combination with all age classes in both data sets.

Discussion

This paper shows how survival analysis can be used to quantify the effect of factors influencing the abortion of flowers and fruit. In this way, effects which were known qualitatively, e.g. “a higher source strength decreases flower and fruit abortion”, can now be quantified. The coefficient for source strength was −0.446 in the temperature experiment when age classes were used. This implies that when source strength increases by $0.1 \text{ g CH}_2\text{O d}^{-1}$, the abortion rate decreases by 4%, given that all other circumstances remain equal.

The cumulative baseline hazard indicates the most vulnerable period for abortion. The steeper the slope, the higher the probability of abortion per degree-day. This period was around 150°Cd after the start of a flower, which is 100°Cd after anthesis. This corresponds to results of Marcelis *et al.* (2004), who reported that most flowers aborted within ten days after flowering (at 20°C). The source and sink strength were quantified as expected: an increase in source strength decreased the abortion rate, while an increasing sink strength increased this rate. Only at very high values of sink strength in the density experiment unexpected results were found. In the density experiment, the influence of source strength is more profound, but in the temperature experiment, the effect of sink strength is stronger (Fig. 6.3). This is related to the treatments in the experiments. Planting density directly affects source strength, and temperature has a strong influence on sink strength.

If age classes were used as covariates (univariate as well as multivariate), some older age classes (around 650°Cd) had a high impact on the abortion rate, while based on physiological knowledge, the impact of those age classes on the abortion rate should be low (Fukumoto *et al.*, 2004; Marcelis *et al.*, 2004).

Table 6.1 Coefficients β and the average percentage change in β when one data point was removed from the data set. For each of the covariates in the analyses with source and sink strength, and the analyses with source strength and age categories, the change in β was obtained when one data point was removed. All changes, both positive and negative, are given as positive percentages.

		Source and sink strength				Indication of presence of fruit			
		Source strength	Source strength ²	Source strength ³	Sink strength	Sink strength ²	Sink strength ³	Position of the flower	Position of the flower
Temperature	Coefficient β	- §	- §	-0.10	1.00	-0.11	0.005	0.35	0.45
	Average deviation (%)	- §	- §	0.016	0.034	0.063	0.084	0.043	0.034
	Coefficient β	-1.20	0.16	-0.007	- §	0.047	-0.0029	0.32	0.33
Density	Average deviation (%)	0.009	0.018	0.303	- §	0.009	0.013	0.021	0.021

See description in results about age classes as covariates

§ Term not included in the analysis

* This is the average value of all age categories, range 0.025% - 0.071%

** This is the average value of all age categories, range 0.011% - 0.037%

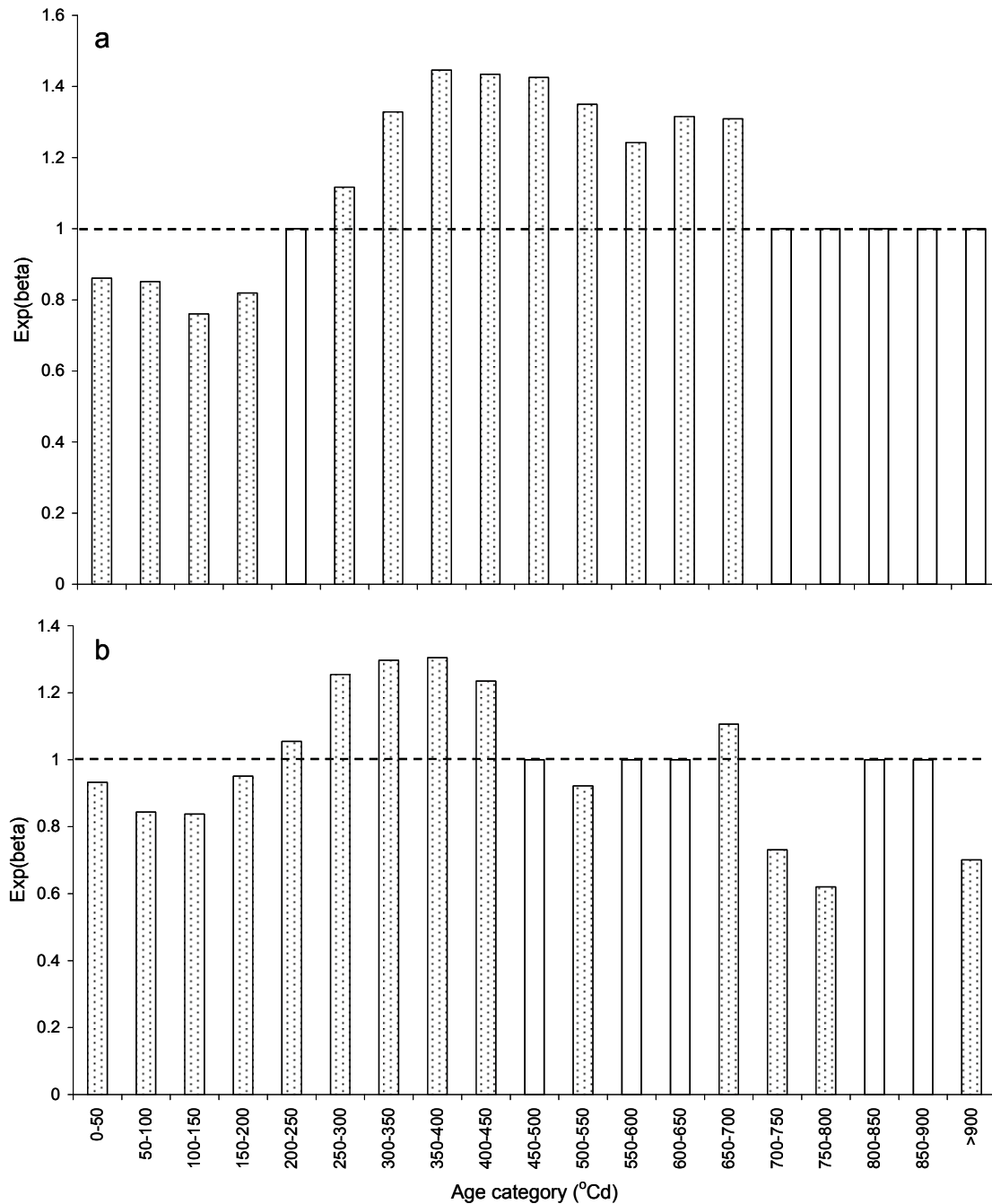


Fig. 6.6 Effect of each age category on the baseline hazard in univariate analyses for the temperature experiment (a) and density experiment (b). Significant age categories ($P < 0.05$) are dotted and non-significant age categories are white. Horizontal dotted line indicates no effect on baseline hazard.

This might be due to the use of the age classes as time-dependent covariates. Time-dependent covariates can easily lead to misinterpretation of the results (Fisher and Lin, 1999). Age classes were therefore also tried as fixed covariates. The number of fruit present in each age class was recorded at the first survival day of each flower. This analysis indicated a high impact on the abortion rate for older age classes as well (data not shown).

Hence, the unexpected results were not caused by the use of time-dependent covariates, but are in the data itself.

Both sink strength and the age classes were not completely independent of source strength. Combinations of very high source strength with low sink strength (presence of only very young and very old fruit), and low source strength with high sink strength (presence of fruit aged around 350°Cd) did not exist. There is also correlation between the age classes themselves. Most fruit were ripe and harvested around 750°Cd, but some fruit took longer to ripen. These very old fruit were present when also strongly competing fruit were present. In the analysis, part of the competition from the fast growing fruit was attributed to the oldest fruit. The correlation between the covariates can lead to unexpected effects at high values of sink strength or older age classes (Fig. 6.3 and 6.6). With fruit pruning, the combination of high source strength with low sink strength could have been obtained, but the combination of low source strength with high sink strength is difficult to obtain.

The timing and the relative magnitude of the age classes were nearly equal to the sink strength of an individual fruit (Fig. 6.6). This means that the potential growth rate of the fruit as a measure for the sink strength, although sometimes criticised (Farrar, 1993), is a good measure. The use of age classes in the analysis had the advantage that the linear assumption between the covariates and the log hazard was not violated. Source and sink strength are often used in relation to abortion (e.g. Alkio *et al.*, 2003; Marcelis *et al.*, 2004).

An important assumption of the Cox model is the proportionality assumption. This implies that the hazard ratio of two subjects with fixed covariates is constant over time. For time-dependent covariates, the ratio does not have to be independent of time, but the ratio between two values of one covariate is still summarised by coefficient β (Therneau and Grambsch, 2000). The proportionality assumption was not met for most analyses. However, for large data sets, the violation of the proportionality assumption does not change the interpretation of the results (Therneau and Grambsch, 2000).

Lag times are often used with time-dependent covariates (Fox, 2002; Kleinbaum and Klein, 2005). The timing of the event is related to a lagged covariate: if the event happens at time t , it is related to the covariate value at time $t-a$, where a is the lag time. This is done because the effect of the covariates does not immediately cause the event. However, in our case the lag time is applied to deduce the event of interest (abortion) from the observed event (abscission). This lag time also influenced the value of the covariates sink strength and age classes, which would not be affected by applying the lag time in the normal way.

The observations on a flower started at anthesis. Some buds, however, aborted before anthesis. This fraction was 0.08 in the temperature experiment and 0.13 in the density experiment. This information could not be included in the analysis and resulted in a slight underestimation of the abortion rate. This was a clear disadvantage of the observations, which started at anthesis of a flower. Dungan *et al.* (2003) gave leaves which abscised before observation a life span of 2 days. As their covariates were not time-dependent, this could be done. For time-dependent covariates, it is difficult to assign the right value of the covariate, as the abscission dates of the buds were not recorded. To overcome this problem, observations should start at bud initiation (about two weeks before anthesis (Erickson and Markhart, 2002)), but this is not feasible in practice.

In conclusion, the abortion rates of reproductive organs can be quantified with survival analysis. The direction of the effect (whether a factor increased or decreased the abortion rate) was correctly estimated, and the analysis revealed that the effect was not linear. Care should be paid to interpretation of the results when correlation between the covariates exist. Comparison of the coefficients of the age classes (Fig. 6.6) with the shape of the sink strength showed that the sink strength is a good approximation of the competitive effect of different age classes. This is an example of information that could only be obtained through survival analysis. Abortion of reproductive organs in sweet pepper was used as an example. The method can equally well be applied to other crops where abortion of reproductive organs occurs, influenced by similar process, e.g. cucumber (Hikosaka and Sugiyama, 2004), soybean (Egli and Bruening, 2006a), and cotton (Pettigrew, 1994). Results of survival analysis can be used in crop simulation models. However, the use of survival analysis is not limited to organ abortion, but could also be used for processes where timing of an event is of interest, like bud break in roses (Marcelis-van Acker, 1994), tillering in wheat (Evers *et al.*, 2006), and shedding of tree branches (Buck-Sorlin and Bell, 1998). The flexibility provided by the use of time-dependent variables allows numerous possibilities for the use of survival analysis.

Appendix Chapter 6

Goodness of fit for other covariates

Apart from source and sink strength as covariates, also other covariates deduced from source and sink strength were used in the survival analysis. The first analysis used source-sink ratio as a covariate (I). Again averages ranging from one to 10 days were tried. The best average source-sink ratio was selected by Akaike's Information Criterion (AIC). Source-sink ratio averaged over 10, respectively 2 days was the best analysis in the temperature and density experiment, respectively. The second analysis was done with the combination of the covariates source and sink strength (II), which is shown in the main text.

Source strength, sink strength and source-sink ratio are plant specific covariates: at a given day, all fruits on the same plant have the same value of the covariate. However, covariates which are fruit specific (e.g. each fruit has a different value of the covariate) might better explain the survival times. Cumulative source-sink ratio (III), calculated individual fruit weight (IV) and the ratio between actual and potential fruit weight (V) were the three fruit specific covariates used. The cumulative source-sink ratio for a fruit was the sum of the daily source-sink ratios since the start of the flower (at the lag time before anthesis). Calculated fruit weight was zero between the start of the flower and anthesis, and increased after anthesis. The daily gain in fruit weight was calculated as the daily source-sink ratio multiplied by the sink strength of the fruit. For the ratio between actual and potential fruit weight, the potential weight of each fruit was based on the daily sink strength value of the fruit. The ratio between calculated fruit weight and its potential fruit weight could not be calculated before flowering (as the sink strength is zero), and the source-sink ratio (truncated to 1 if the source-sink ratio is higher than 1) was used instead.

Comparing the analysis with the five covariates revealed that overall, source and sink strength could best explain the observed abortion times .

Table A6.1 The AIC and the total rank sum of the analyses with different covariates. The lowest AIC within an experiment indicated the best analysis and was given rank 1, the worst analysis had rank 5. Total rank sum is the sum of the two ranks (one in each experiment) of a covariate.

Covariate	Temperature experiment	Density experiment	Total rank sum
I. Source-sink ratio (moving average)	9915	26554	6
II. Source and sink strength (moving average)	9816	26546	3
III. Cumulative source-sink ratio	9878	26602	6
IV. Calculated fruit weight	10032	26973	10
V. Ratio actual/potential fruit weight	9943	26494	5

Chapter 7

Stochastic simulation of flower and fruit abortion with a mechanistic model

Abstract

Most crop simulation models are deterministic, i.e. only one output value is obtained. In reality, variation in plant growth and development is observed. In this study, stochasticity is introduced into a mechanistic model. Fruit abortion in sweet pepper is used as a case study, as fruit abortion is highly variable in time as well as between plants. The abortion function was derived from survival analysis. Observations on dates of anthesis, abortion and harvest were obtained in a planting density experiment (2.5, 3.8 and 5.0 plants m⁻²). For each flower, time between anthesis and abortion, harvest or end of the experiment was related to source and sink strength. The results of this analysis were incorporated in a mechanistic model for crop growth, development and yield of sweet pepper. After calibration, the model was able to simulate observed fruit set patterns. Validation with another planting density experiment resulted in the observed fruit set pattern, except for a period of low fruit set at the beginning of the cultivation. In both data sets, the between-plant variation of fruit set in the simulations was lower than observed. Validation with two other data sets gave reasonable results. A case study in which the source strength was kept constant showed that a variable source strength synchronises the fruit set pattern of the plants.

Introduction

Crop growth models have become widely used in research and education (Gary *et al.*, 1998). These models are predominantly deterministic. Applying a specific input (climate, crop parameters) results for each output variable in a single value per time step, i.e. without confidence limits for the output variables. For the assessment of climate variability, crop simulation models are often run with stochastic weather inputs, which leads to variation in the output variables (e.g. Semenov *et al.*, 2009). However, the growth of the crop is still simulated deterministically. Models typically simulate average plants neglecting the existing variation among plants. Regarding the growth of individual organs, it is assumed that each organ obeys in exactly the same way to internal or external factors. In reality, inter-plant and inter-organ variation is observed under apparently identical conditions. In some simulation models variation in a process is incorporated. Examples thereof are truss appearance rate in tomato (Pearson *et al.*, 1996), number of buds and bud break in kiwi (Agostini *et al.*, 1999), and abortion of cotton bolls (Lieth *et al.*, 1986).

A sweet pepper crop exhibits fluctuations in fruit set: periods of high fruit set alternate with periods of low fruit set (Heuvelink *et al.*, 2004). These fluctuations occur due to abortion of buds, flowers and young fruits. In short, abortion of organs can be induced by low supply of assimilates (source strength), competition from other organs (sink strength) (Marcelis *et al.*, 2004), high ambient temperatures (Aloni *et al.*, 1994), and shortage of water and nutrients (Guilioni *et al.*, 2003). Additionally, hormonal dominance of one organ over another plays a role (Bangerth, 1989). Under high radiation and ample supply of water and nutrients, competition between fruits and dominance hierarchy of fruits are the most important factors influencing abortion. When fruits are nearly mature, new flowers have the opportunity to set and grow into a harvestable fruit. In contrast, the presence of fast growing fruits (around three weeks old) results in abortion of almost all flowers and young fruits (Marcelis *et al.*, 2004). This mechanism leads to alternating periods of high and low fruit set. Apart from fluctuations in time, also differences exist in fruit abortion between plants; positions where fruit abort differ slightly from plant-to-plant. Regarding this variation in fruit abortion, in time as well as between plants, a crop simulation model with stochastic flower and fruit abortion is more realistic than deterministic simulation of fruit abortion.

The aim of this Chapter is to introduce stochasticity for fruit abortion in a mechanistic model. In a model with a stochastic component for fruit abortion, a fruit might set in one simulation run and abort in another simulation run, even under identical circumstances. Whether or not a fruit aborts influences the probability of abortion for future fruits. We hypothesize that a simulation model with stochastic fruit abortion based upon source and sink strength is able to accurately simulate fruit set patterns and the plant-to-plant variation in fruit set observed in experiments. Survival analysis was used to obtain a function which enables stochastic simulation of fruit abortion timing (Chapter 6). The model is validated with three data sets. Moreover, a case study on the role of source strength in the synchronisation of fruit set fluctuations is conducted.

Model description

The simulation model is based on the model INTKAM (Gijzen, 1994; Marcelis *et al.*, 2006), adapted for the sweet pepper crop. INTKAM is able to simulate accurately the growth of the total crop and the fruit yield of sweet pepper over time (Marcelis *et al.*, 2006). The model simulates the formation of organs, leaf area expansion and dry and fresh weight growth of the different organs. Rate of leaf and flower formation as well as leaf area expansion are determined by temperature (Marcelis *et al.*, 2006). Interception of photosynthetic active radiation (PAR) is calculated for a multi-layered uniform canopy (Goudriaan and Van Laar, 1994), assuming a spherical leaf angle distribution. Leaf gross photosynthesis is calculated with the biochemical model of Farquhar *et al.* (1980) at five depths in the canopy. Canopy gross photosynthesis is computed from leaf photosynthesis with the Gaussian integration method (Goudriaan and Van Laar, 1994). Assimilates available for growth are calculated as the gross assimilate production minus the maintenance respiration. The maintenance respiration depends on the type and weight of the organ, as well as the temperature. The assimilates are divided between fruits and vegetative organs based on the concept of sink strengths (Marcelis, 1996): the amount of assimilates partitioned to one fruit equals the total amount of available assimilates multiplied by the fruit's share in the total sink strength. The sink strength of a fruit is a function of its age (expressed in temperature sum after anthesis). It is calculated by a set of equations: a Richards function describing the fresh weight depending on fruit age and a Ricker function for converting fresh weight into dry weight depending on fruit age (Chapter 3). The sink strength is the derivative of the multiplication of these two functions. Temperature had no effect on the sink strength of fruits: the potential fruit size was the same, regardless of temperature. The vegetative sink strength increased with increasing temperature (Chapter 6: density experiment). Conversion of assimilates (g CH₂O) to dry weight is computed by a factor representing the assimilate requirement per unit dry weight of the organ. In the model fruits are harvested at a specified temperature sum after anthesis. This stage can vary with the desired fruit colour (green or red).

Derivation of the function for flower and fruit abortion

Abortion was simulated using the results of survival analysis (Chapter 6, density experiment; Kleinbaum and Klein, 2005). With survival analysis, the survival times of flowers and fruit were related to factors influencing abortion, the so-called covariates. The result of survival analysis exists of a cumulative baseline hazard and a set of coefficients. The baseline hazard is the probability of abortion per unit time assuming that all covariates are zero. The coefficients quantify the effect of the covariates. The baseline hazard is multiplicatively influenced by the covariates, the latter represented by an exponential function of a linear combination of the covariates (equation 7.1).

$$h_{f(t_f)}(t_c, X) = h_{0,f(t_f)}(t_c) * \exp\left[\sum_{i=1}^n \beta_i X_i(t_c)\right] \quad \text{equation 7.1}$$

Here, $h_{f(t_f)}(t_c, X)$ is the probability of abortion per unit time (abortion rate) at calendar time t_c of fruit f with age t_f as influenced by fixed or time-dependent covariates $X_i(t_c)$

($i=1,...,n$): $h_{0,f(t_f)}(t_c)$ is the baseline probability of abortion at calendar time t_c of fruit f with age t_f , $X_i(t_c)$ are the values of the covariates at time t_c and β_i the coefficient quantifying the effect of the covariate X_i .

In a density experiment with three planting densities, 2.5, 3.8 and 5.0 plants m^{-2} , flowering, abscission and fruit harvest were observed (for further experimental details: see calibration data). The observed survival time for a flower or fruit was from anthesis till harvest, abscission or the end of the experiment. To correct for the lag time between abortion and abscission, the analysed survival time of all flowers started 50°Cd before anthesis and ended, for aborted flowers and fruits, 50°Cd before abscission. For fruits which did not abort (censored observations), the survival time ended at their harvest time or when the experiment was ended. The covariates used in the survival analysis were the time-dependent covariates source strength, sink strength, and the fixed covariate position of the fruit on a plant (whether it was on the main or on the side branch, coded 0 and 1 respectively). Source strength was averaged over the past two days and sink strength over the past 10 days. The effect of source and sink strength was quantified by a polynomial function, with 1st, 2nd and 3rd order terms for source strength and 2nd and 3rd order terms for sink strength. The estimated cumulative baseline hazard had a sigmoid shape (Chapter 6, Fig. 6.5). A Gompertz function was fitted through the cumulative baseline hazard and its derivative with respect to time was taken to obtain the baseline hazard. Fig. 7.1 shows the baseline hazards for the analysis with and without position of the flower, and the effect of source strength and sink strength thereon. As the source strength increases, the abortion rate is lower. The opposite holds for sink strength. The polynomial functions for source and sink strength did not differ between the analysis with and without position of the flower included. The coefficient for flowers on the side shoot was 0.32, implying that the hazard was 1.38 times higher for a fruit on the side shoot than a fruit on the main shoot (when all other circumstances are equal).

Simulation of flower and fruit abortion

As the analysed survival times of the flowers and fruits started 50°Cd before anthesis, the appearance (start) of a flower in the simulation was at a flower age of 50°Cd before anthesis. In the 50°Cd between the start of a flower and anthesis, the flower had no sink strength and did not accumulate fruit weight. The flower had a sink strength and started to accumulate dry matter when it was older than 50°Cd. For each flower and fruit in the critical period, from 50°Cd before anthesis till 150°Cd after anthesis, the daily abortion rate is calculated. The baseline abortion rate depends on the age of the flower/fruit, and was given per degree-day (Fig. 7.1). It was integrated over the degree-days per day (daily average temperature minus the base temperature 10°C) to obtain the baseline abortion rate per day. Subsequently the effects of source strength, sink strength and, if applicable, position of the flower, on the abortion rate were incorporated in the abortion rate. For stochastic simulation a random number between 0 and 1 was drawn and compared to the calculated abortion rate. If the random number was smaller (larger) than this rate, the flower aborted (or not). The presence of each fruit influences the sink strength, one of the covariates in the hazard function, and therefore it influences the abortion rate of other fruits. Each simulation of the model represents a sweet pepper crop with similar plants.

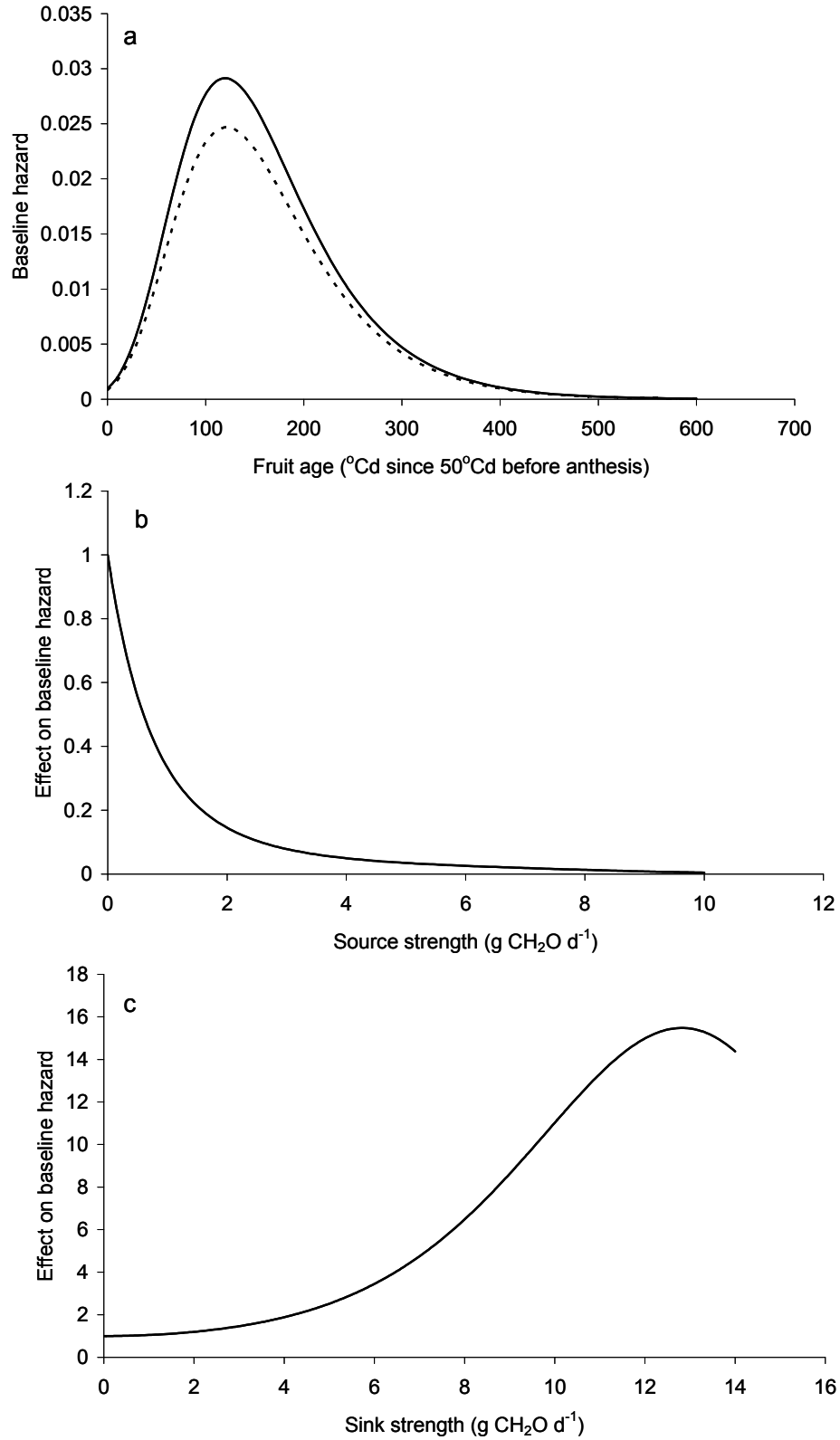


Fig. 7.1 The baseline hazard (probability of abortion per degree-day) for the analysis without position of the flower (solid line) and with position of the flower (dotted line) (a), the effect of source strength on the baseline hazard (b) and the effect of sink strength on the baseline hazard (c). Effect on the baseline hazard is given as $\exp(\beta_i X_i(t_c))$ from equation 7.1, where $X_i(t_c)$ is source strength (b) or sink strength (c).

Calibration and validation data

The data used for calibration were the same data as used in the survival analysis. Three additional experiments were used for validation of the calibrated parameters. Plants were grown on rockwool in all experiments except the second validation data set, where the plants were growing in coconut fibre. Water and nutrient were supplied by trickle irrigation. Plants were pruned to two stems per plant, except the third validation data set, where plants were pruned to three stems per plant.

Calibration data: Sweet pepper plants *Capsicum annuum* ‘Red Spirit’ (Enza seeds, Enkhuizen, the Netherlands) were grown in a Venlo-type glasshouse in Wageningen from December 2001 to early September 2002 at planting densities of 2.5, 3.8, and 5 plants m⁻². Plants were grown with two main stems per plant. The realised average daily temperature during the experiment was 21.6°C ± 2.0 (mean ± sd). The average global outside radiation was 11.6 MJ m⁻² d⁻¹ ± 7.7 (mean ± sd). Transmission of the greenhouse was 65%. The average CO₂ concentration during the day was 430 µmol mol⁻¹ and the average daily relative air humidity was about 75%. Red fruits were harvested twice a week. Observations were done on 14 plants per density. This calibration data was the same data which was analysed with survival analysis.

Validation data set 1: Sweet pepper plants of cultivar ‘Mazurka’ (Rijk Zwaan, De Lier, the Netherlands) were grown in a Venlo-type glasshouse in Wageningen, the Netherlands, from January till July 1997 at planting densities of 1.56, 3.12 and 4.63 plants m⁻². Plants were grown with two main stems per plant. The realised average daily temperature during the experiment was 21.3°C ± 1.5 (mean ± sd). The average global outside radiation was 11.3 MJ m⁻² d⁻¹ ± 7.6 (mean ± sd). Transmission of the greenhouse was 65%. The average CO₂ concentration during the day was 450 µmol mol⁻¹. Fruit were harvested red. Observations on fruit set were done on 12 plants per density.

Validation data set 2: Sweet pepper plants of cultivar ‘Spider’ (Enza zaden, Enkhuizen, the Netherlands) were grown in a semi-closed greenhouse at the Improvement Centre in Bleiswijk, the Netherlands, from December 2007 till October 2008 at a planting density of 3 plants m⁻². Plants were grown with two main stems per plant. The realised average daily temperature during the experiment was 21.1°C ± 1.4 (mean ± sd). The average global outside radiation was 12.0 MJ m⁻² d⁻¹ ± 7.7 (mean ± sd). Transmission of the greenhouse was 70%. The average CO₂ concentration during the day was 844 µmol mol⁻¹ and the average daily relative air humidity was about 80%. Fruit were harvested red. Fruit set was observed in two fields of 2 m², which were renewed halfway the experiment.

Validation data set 3: Sweet pepper plants of cultivar ‘Orange Glory’ (The Ruiter Seeds, Bergschenhoek, the Netherlands) were grown in a commercial greenhouse in Kwintsheul, the Netherlands from December 2008 till October 2009 at a planting density of 2.4 plants m⁻². Plants were grown with three main stems per plant. The realised average daily temperature during the experiment was 20.9°C ± 1.3 (mean ± sd). The average global outside radiation was 11.3 MJ m⁻² d⁻¹ ± 7.4 (mean ± sd). Transmission of the greenhouse was 70%. The average 24h CO₂ concentration was 707 µmol mol⁻¹. Fruits were harvested orange.

Simulations

The key output variable was fruit set. A fruit was set when it survived a critical period of 150°Cd after anthesis. Weekly fruit set of a plant was calculated as the number of set fruit in one calendar week. The average weekly simulated fruit set was the fruit set of a specific week averaged over all simulated plants. The between-plant variation of the fruit set within a week is defined as the standard deviation of the fruit set in that week. For each simulated and observed data set, the average simulated fruit set pattern in time was obtained, as well as the total number of set fruit per plant and the weekly variation of fruit set within a plant. Weekly variation of fruit set within a plant was defined by the coefficient of variation (C.V.), which is the standard deviation of the weekly fruit set of a plant (1 simulation run) divided by the average weekly fruit set of that plant. Comparisons were made between observed and simulated values.

In general, the variation around the average is represented by the standard error, i.e. the standard deviation divided by the square root of the number of observations. As the optimum number of simulations and the number of plants used for observations differed considerably, the standard error was not an appropriate way to compare the between-plant variation of weekly fruit set between the simulations and the observations. The error bars in the graphs with average weekly fruit set plotted against time are a quarter of the standard deviation. In this way, the variation between the simulations and the observations can be fairly compared.

Establishing the optimum number of simulation runs

As the fruit set pattern varied between simulations due to the stochastic fruit abortion, it was necessary to establish the number of simulations which resulted in a stable fruit set pattern. Initially, 500 simulation were done with the settings of the 5.0 plants m⁻² treatment from the calibration data set. The average fruit set over 500 plants was calculated for each week. Next, random samples were drawn from these 500 simulations. Sample sizes were 1, 5, 10, 25, 50, 100, 200, 300 and 400 simulation runs and each sample size was drawn 3 times. The average fruit set in each week was calculated for each subset. The root mean square error (RMSE, equation 7.2) was calculated between the weekly fruit set of the 500 simulations and the weekly fruit set of the subset.

$$RMSE = \sqrt{\left[\frac{1}{n} \sum_{i=1}^n (F_i - S_i)^2 \right]} \quad \text{equation 7.2}$$

F_i is the average number of set fruits per plant in week i in the full data set, S_i is the average number of set fruits per plant in week i in the subset, and n is the number of weeks with fruit set (here 27).

The RMSE's were plotted against sample size. The optimum number of simulations is defined to have an average RMSE less than 0.1. A RMSE of 0.1 represents an average absolute difference between the weekly fruit set in the subsample and the weekly fruit set in the 500 simulation of 0.1 fruit week⁻¹.

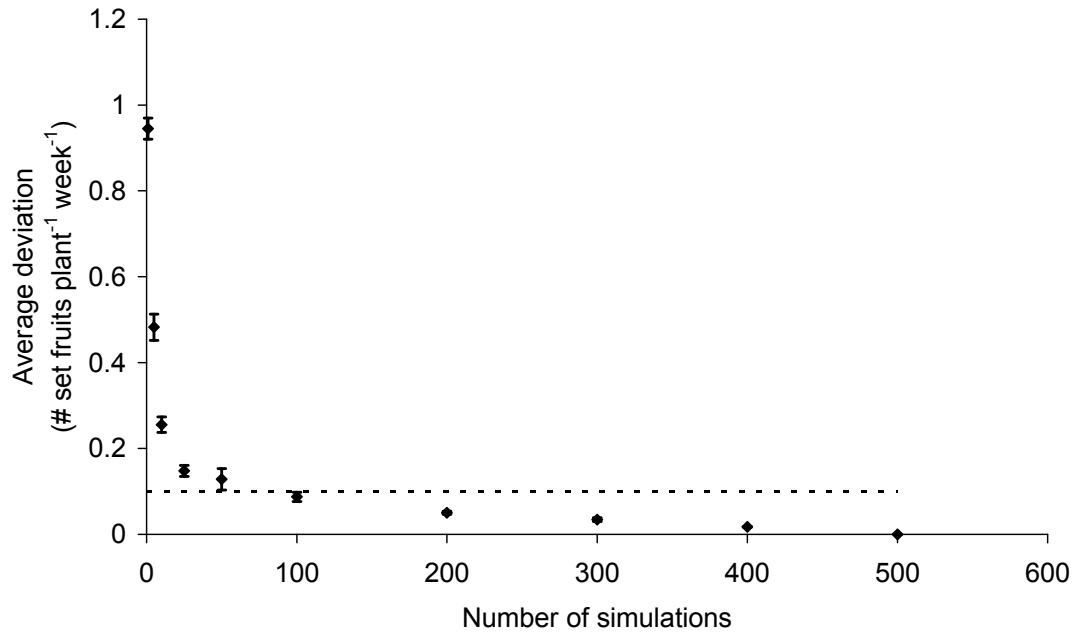


Fig. 7.2. Average deviation in weekly fruit set between the average of 500 simulations and a subset of these 500 simulations. Each data point consist of 3 subsamples of the same size. Error bars indicate standard error of the mean. Dotted line indicates the average deviation of 0.1.

Calibration procedure

The abortion parameters were calibrated on the data set which was analysed with survival analysis. First, the simulated total dry matter production was calibrated on the measured total dry matter production by correcting the plant growth rate. Part of the flowers aborted before anthesis in the experiment, especially in the first two weeks of the cultivation. The abortion rate for flowers in the first two weeks of simulation was corrected for the abortion of buds.

Then, 100 simulations were done for each density of the calibration data. The average number of set fruit in each week of 100 simulations was compared to the averaged observed number of set fruit in each week of the 14 plants. The fruit set pattern was reasonably simulated for the highest planting density, 5.0 plants m⁻², but poor for the other two densities, 3.8 and 2.5 plants m⁻² (data not shown). Analysis of the simulation results showed that the effect of sink strength at high values was not strong enough, i.e. the abortion rates at high sink strength (>6 g CH₂O d⁻¹) were too low. At source strengths higher than 6 g CH₂O d⁻¹, the decrease in abortion rate was too low. The coefficients were adapted to yield a fruit set pattern more close to the observed fruit set pattern. The resulting relations are shown in Fig. 7.1b,c.

Case study

The fluctuations in fruit set result in fluctuations in fruit yield, which is undesirable. The sweet pepper crop is planted in December and cultivation continues till the following November. The leaf area as well as radiation levels are increasing in the first months of the cultivation. The effect of this combined increase of leaf area and radiation on fruit set is investigated. Scenario studies were conducted, consisting of simulations with a constant

leaf area index (LAI) and measured environmental data (radiation, temperature, humidity and CO₂ concentration), and simulations with constant source strength. The average fruit set pattern as well as the between-plant variation of weekly fruit set were examined to investigate if this parallel increase in leaf area and radiation levels, resulting in increased source strength, are the cause of synchronised fruit set patterns between the plants. The constant LAI was set at 2.5. The environmental data were from the second validation data set. The constant source strength was the average of the source strength in the simulation with constant LAI and measured environmental data (5.9 g CH₂O d⁻¹). Planting density was 3 plants m⁻² with two stems per plant.

Results

Optimum number of simulated plants

The fruit set pattern differed per simulation. The difference in average simulated fruit set pattern between 500 simulations and a subset of these 500 simulations decreased quickly when the subset increased from one to 25 simulations (Fig. 7.2). Above 25 simulations the decrease in deviation was much smaller. At 100 simulations, the average weekly deviation was less than 0.1 fruits plant⁻¹ week⁻¹, and this number was therefore considered as sufficient to obtain a stable simulation pattern.

Calibration

The simulations with the calibrated parameters gave a reasonable simulation of the observed fruit set pattern (Fig. 7.3a-c). However, the total number of set fruit was overestimated and the weekly variation of fruit set within a plant (C.V.) was underestimated (Table 7.1). The overestimation of fruit set was for a large part caused by the overestimation of fruit set around week 9. However, to improve the simulated fruit set in this part of the cultivation period, a different set of parameter combinations was needed, which did not perform accurately in the other weeks (data not shown). The standard deviation of weekly fruit set in the simulations was lower than the observations (Fig. 7.5).

If the position of the flower on a plant was included the simulation of fruit set did neither improve nor worsen (Fig. 7.3a-c). This covariate was therefore not used for the validation.

Table 7.1 Number of set fruits per plant and the within-plant variation of weekly fruit set (C.V.) in the observations and the simulations (after calibration) of the calibration data set. Simulations were done with and without including the position of the flower (whether it was on the main or on the side shoot). Number between brackets represent the standard deviation.

		Data	Simulation			
			Without position of flower		With position of flower	
	#	C.V.	#	C.V.	#	C.V.
5.0	20 (3.4)	1.47 (0.25)	30 (2.4)	1.06 (0.12)	30 (2.3)	1.12 (0.13)
3.8	21 (4.7)	1.47 (0.28)	33 (2.4)	0.92 (0.13)	33 (2.3)	0.99 (0.13)
2.5	31 (4.4)	1.27 (0.21)	40 (2.7)	0.87 (0.11)	40 (2.3)	0.90 (0.11)

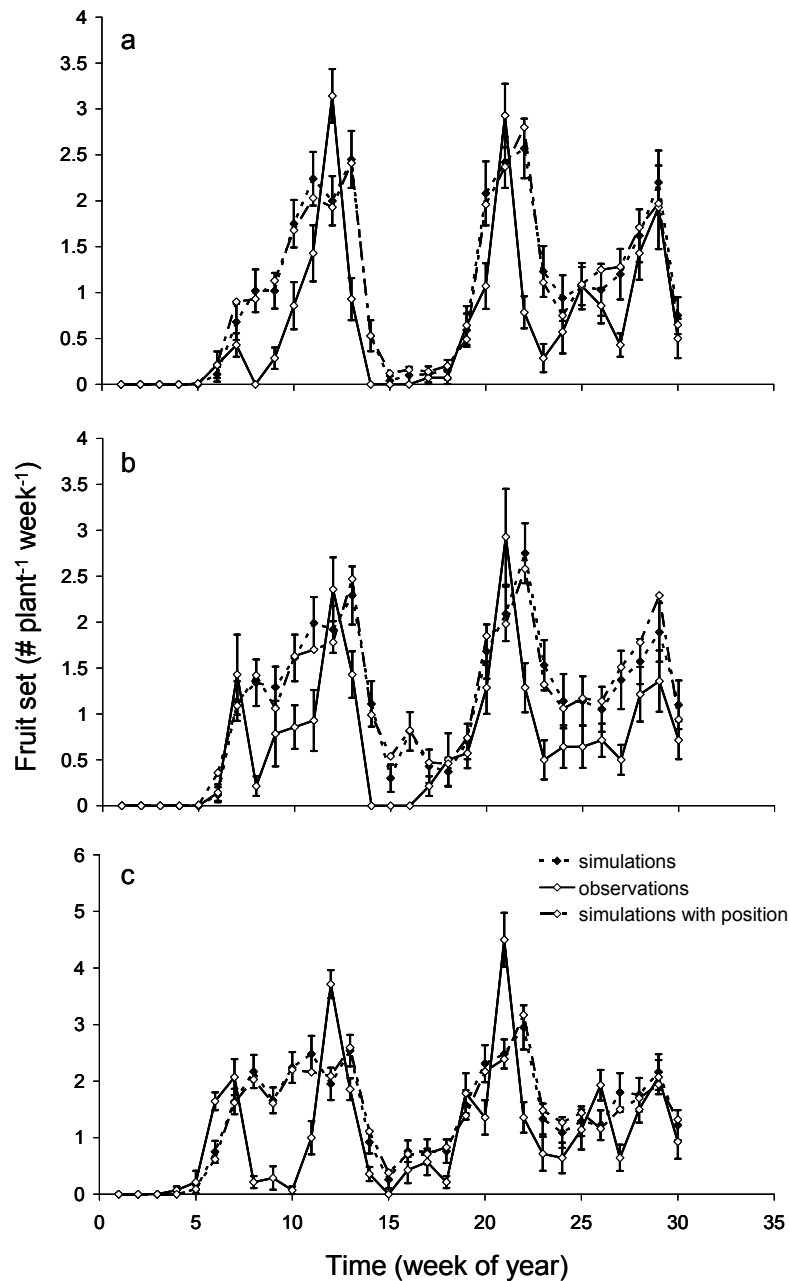


Fig. 7.3 Simulations after calibration. Calibration data were obtained from an experiment with the cultivar 'Red Spirit' at three densities with two stems per plant. Dates represent anthesis dates of non-aborting fruits. Error bars represent standard deviation divided by 4. Simulation is done with and without the covariate position of the flower, which indicates whether the flower or fruit is on the main shoot (=0) or on the side (=1) shoot. a) 5.0 plants m^{-2} , b) 3.8 plants m^{-2} , c) 2.5 plants m^{-2} .

Validation

Data set 1; On average, the fruit set pattern observed in the experiment was also observed in the simulations, although the low fruit set around week 12 was not simulated in all three densities (Fig. 7.4a-c). In the highest density (4.63 plants m^{-2}), the low fruit set in week 21 was not simulated. The total number of set fruits and the variation in weekly fruit set were almost correctly simulated in the highest plant density, but the variation in weekly fruit set

was underestimated in the intermediate density, and the total fruit set was overestimated and the variation in weekly fruit set was underestimated in the lowest planting density (Table 7.2). The standard deviation of the fruit set per week was underestimated in all simulations: in the observations, the standard deviation of the fruit set per week increased with increasing average fruit set per week, but in the simulations, it stayed more or less constant around 1.2 after an initial increase (Fig. 7.5).

Data set 2; Fruit set in the first peak was correctly timed, but the peak was wider than in the observations (Fig. 7.6). The timing of the second peak in fruit set was two weeks too late. Fruit pruning was applied after the first and second peak of fruit set, which might cause the discrepancy between the observed and simulated fruit set pattern in the first two peaks. The third peak was correctly timed and of the same magnitude, but the peak in week 31 lacked in the simulations. The total number of set fruits and the weekly variation of fruit set within a plant (C.V.) were slightly overestimated (Table 7.2).

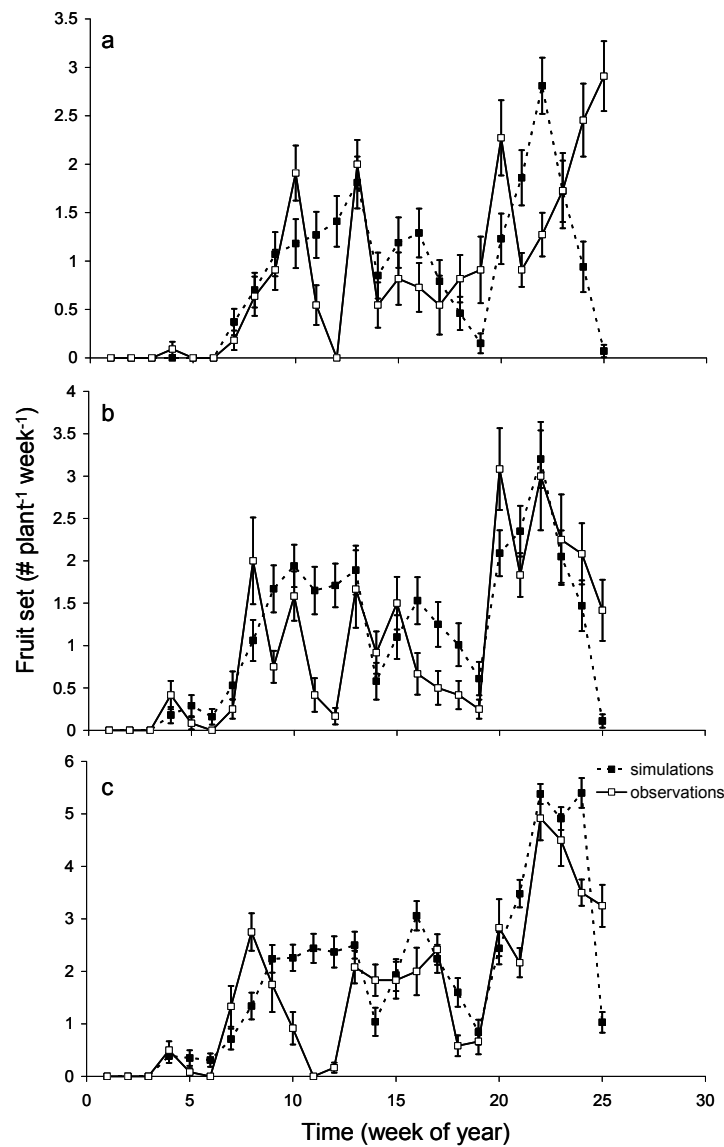


Fig. 7.4 Simulated and observed fruit set pattern of the first validation data set. Data were obtained from an experiment with the cultivar ‘Mazurka’ at three densities with two stems per plant. Error bars represent standard deviation divided by 4. a) 4.63 plants m^{-2} , b) 3.12 plants m^{-2} , c) 1.56 plants m^{-2} .

Data set 3; This data set was simulated assuming 2 stems per plant and a planting density of 1.5 times the original density. In this way the number of stems per square meter was similar. There was an overshoot of fruit set in the first weeks of the cultivation, which resulted in a lower simulated peak at week 17 (Fig. 7.7). The pattern between week 18 and 25 is correctly simulated, but overestimated. The total number of set fruits and the weekly variation of fruit set within a plant (C.V.) were overestimated (Table 7.2).

Case study

A constant leaf area index but normal seasonal environmental data resulted in a fluctuating pattern of fruit set (Fig. 7.8a). Simulation with constant source strength resulted in a synchronized first peak in fruit set, but after this peak strong fluctuations in fruit set were absent (Fig. 7.8b). Individual plants in this simulation exhibited non-synchronised fluctuations, as seen in three randomly drawn plants (Fig. 7.8c).

Discussion

This paper shows that the calibrated survival function for the abortion of sweet pepper flowers and fruits is able to simulate fluctuations in fruit set patterns which resemble the observed fruit set patterns. The between-plant variation of fruit set, per week as well as in total, was in general smaller in the simulations than in the observations (Fig. 7.5, Table 7.1). The higher variation in the observed fruit set might be due to circumstances not accounted for in the model. This can be plant-to-plant differences in e.g. anthesis dates, leaf area and photosynthesis, and/or local differences in climate, e.g. radiation and temperature. In the survival analysis, a frailty term was fitted as well, which accounts for differences in abortion rates between the plants (Chapter 6).

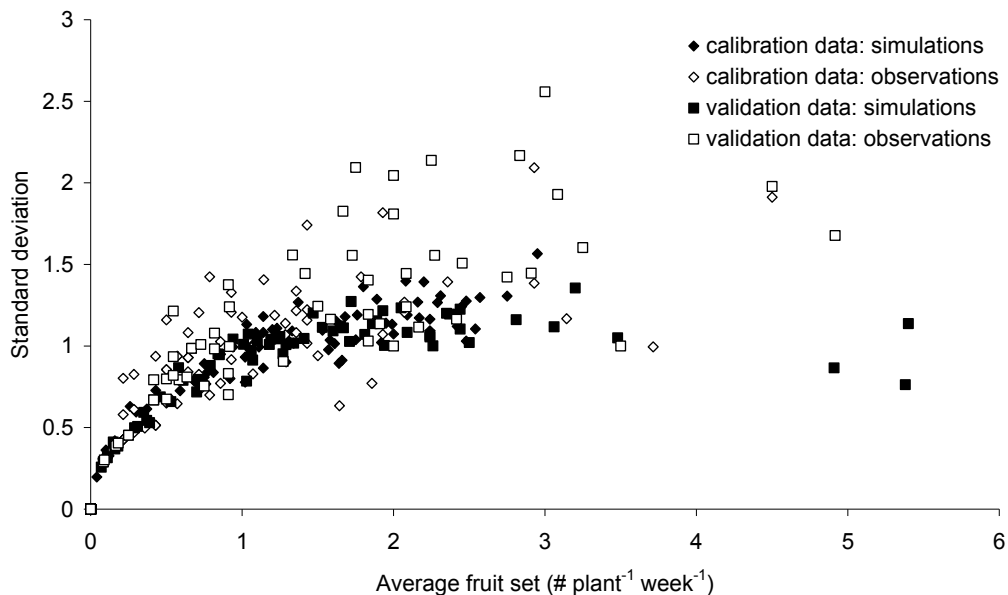


Fig. 7.5 Standard deviation of the weekly fruit set vs. the weekly fruit set of the two density experiments (calibration data set and first validation data set). Average and standard deviations are based on 100 simulations, on 14 plants (calibration data) or 12 plants (validation data).

This term was not yet used in the simulations. Simulation with the frailty term, which slightly increases or decreases the baseline hazard, is likely to increase the variation in the simulated fruit set pattern. In both planting density experiments, there was a period of low fruit set in the observations which was not simulated. This caused high overestimation in the number of set fruits. When the simulation of fruit set started later (after the first peak), the pattern was perfectly simulated. Further analysis of the simulation results showed that a combination of low source strength with high sink strength did not result in the right level of fruit abortion.

The model simulations not only resulted in plant-to-plant differences in the number of fruits per plant and the timing of fruit set per plant. Plant-to-plant differences also existed in the simulated total dry matter production and partitioning of dry matter into the fruits (data not shown). Difference in total dry matter production was due to the variation in the number of fruits, which affected the maintenance respiration and hence the amount of assimilates available for plant growth. Individual fruit weight varied within as well as between the plants, due to variation in source and sink strength during fruit growth.

Stochastic simulation of fruit set made it possible to observe effects which would be unnoticed in a deterministic model, like the asynchronisation of fruit set under constant source strength. The average result of simulations with a constant source strength showed no strong fluctuations in fruit set, but fluctuations were present in each individual plant. This means that the fluctuations in source strength occurring in normal cultivation synchronises the fluctuations of the fruit set patterns between the plants, but synchronisation is absent when source strength is constant. It implies that asynchronisation of the fruit set pattern, needed to obtain a constant fruit yield, is difficult to obtain in practice, as the driving force of the source strength, variability in weather data, is always present. This example was a demonstration of a hypothetical situation which cannot be realized in practice, but it is interesting from a theoretical point of view. Other possibilities of the use of stochastic simulation models are assessing the variability in target output variables under certain circumstances, sample size of experiments and conducting virtual experiments which would be unfeasible in practice due to a large number of treatments (Meyer *et al.*, 2009a).

Table 7.2 Number of set fruits per plant and the within-plant variation of weekly fruit set (C.V.) of the observations and simulations of the validation data sets. The calibrated parameter values were used in all data sets. Number between brackets represent the standard deviation.

	Treatment	data		simulation	
		#*	C.V.	#	C.V.
Data set 1	4.63	22 (1.9)	1.28 (0.10)	21 (2.0)	1.18 (0.13)
	3.12	25 (8.2)	1.31 (0.29)	28 (2.1)	0.99 (0.13)
	1.56	40 (6.2)	1.04 (0.12)	48 (3.3)	0.83 (0.07)
Data set 2	-	142 (3.8)	0.96 (0.06)	160 (12.3)	1.08 (0.08)
Data set 3	-	172 (-)	0.54 (-)	213 (3.2)	0.82 (0.09)

* Numbers of fruit in data set 1 are given per plant, in the other two per m².

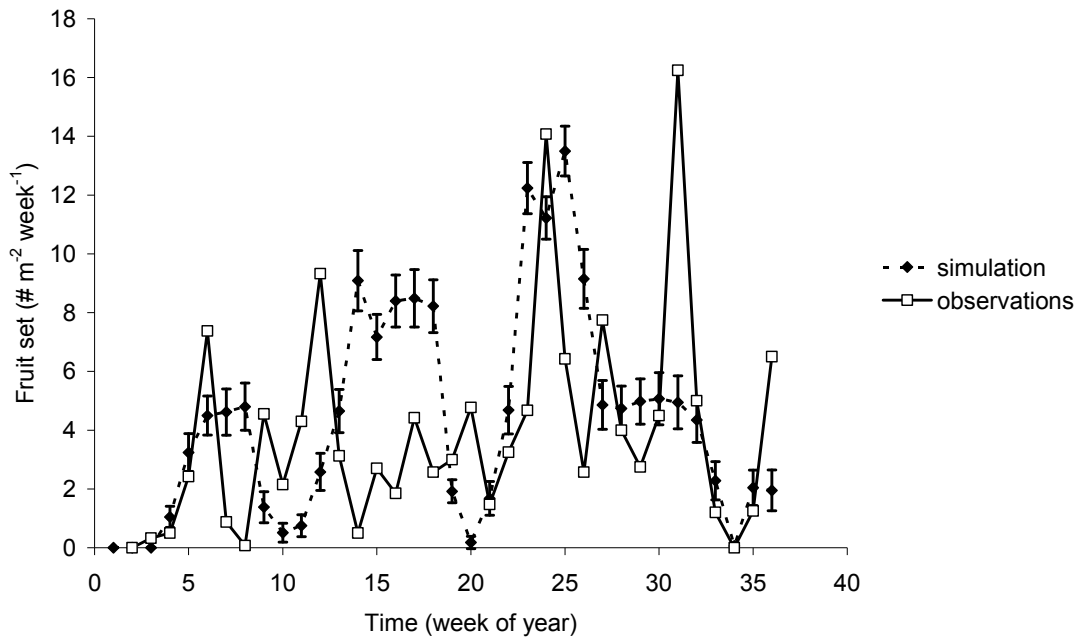


Fig. 7.6 Simulated and observed fruit set pattern in validation experiment 2. Data were obtained from an experiment with the cultivar ‘Spider’ at a density of 3 plant m⁻² with two stems per plant. Error bars represent standard deviation divided by 4.

Variation in simulation output is obtained by incorporating a function which calculates a probability rate for fruit abortion. It makes stochasticity an intrinsic part of the model. Another possibility to make stochasticity an intrinsic part of the model is to assume a specific theoretical distribution for process rates and initial conditions, like the number of buds, spear induction rate and spear growth rate in asparagus (Graefe *et al.*, 2010). When a parameter is a proportion, stochasticity can be obtained by comparing the proportion with a random draw from a uniform distribution between 0 and 1. The proportion can be either calculated depended on some variables or constant (Meyer *et al.*, 2009b). The use of stochastic weather data, artificial time series of weather data which are based on the statistical characteristics of observed weather at a certain location, is often used for assessing the role of climate variability on crop yield (e.g. Lawless and Semenov, 2005). However, stochasticity in this case is not mechanistically built into the model: variation results from the variation in input data.

Abortion is modelled as a function of source and sink strength. These are the most used factors regulating abortion in other models, although sometimes formulated differently, like source-sink ratio (e.g. Lieth *et al.*, 1986; Bertin and Gary, 1993) or the difference between source and sink strength (Buwalda *et al.*, 2006). Source and sink strength as individual covariates fitted better than source-sink ratio in the survival analysis (appendix Chapter 6). Source strength incorporates the effect of the amount of light, leaf area, CO₂ level and temperature on the production of assimilates. Sink strength quantifies the effect of the number and age of the competing fruits, as well as temperature, on the abortion process. Together, source and sink strength could generate the timing of the fluctuations in fruit set, although the amplitude of the fluctuations was not always correct. The most important factor not accounted for was the hormonal dominance of one fruit over another (Bangerth, 1989).

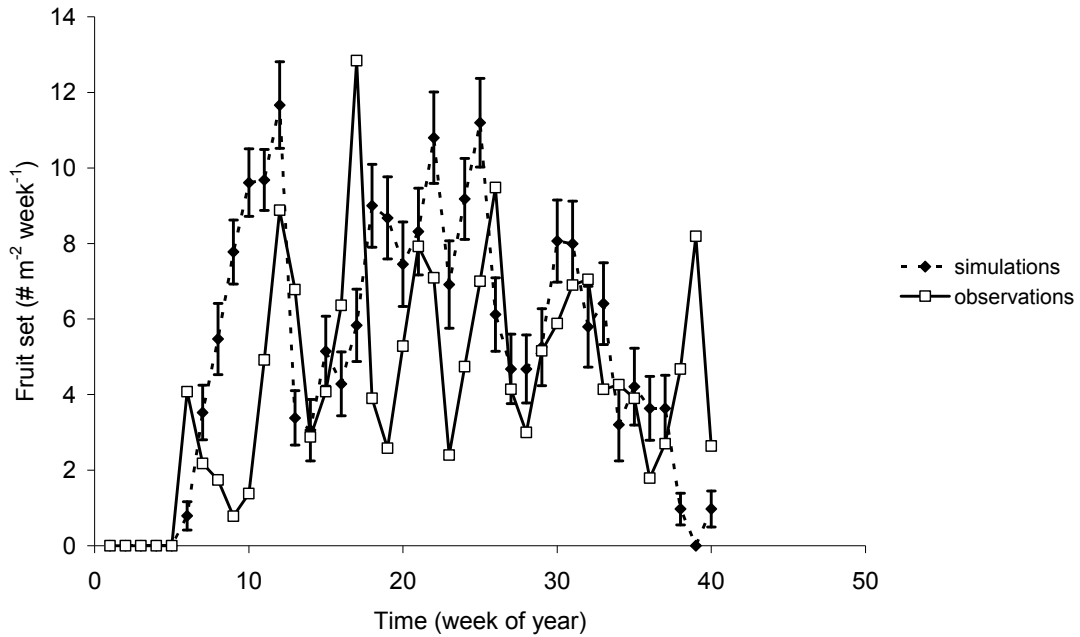


Fig. 7.7 Simulated and observed fruit set pattern in validation experiment 3. Data were obtained from an experiment with the cultivar ‘Orange glory’ at a density of 2.4 plants m^{-2} with 3 stems per plant. Error bars represent standard deviation divided by 4.

Some experimental results on organ growth and development could not be explained by source-sink relationships alone (Marcelis *et al.*, 2004; Pallas *et al.*, 2010), indicating that hormones play an additional role. Prusinkiewicz *et al.* (2009) and Yang and Midmore (2009) developed simulation models which simulate growth and development of plants based on the production and flow of auxins and cytokinins. These models demonstrate how the principle of dominance of one organ over the other and the vascular development due to hormonal transport can lead to differences in growth and developmental patterns. Integration of the hormonal and assimilate concept is a major challenge in plant and crop modelling. Temperature is an important variable playing a role in abortion. It was assumed that in the occurring range of average daily temperatures, temperature influenced abortion via source and especially sink strength. Additional abortion due to higher temperatures can be modelled as well (Challinor *et al.*, 2005). Although the analysis of flower and fruit abortion was improved when the position of the fruit on the plant was added (Chapter 6), it did not improve the simulation of fruit abortion (Fig. 7.3). Adding the position of the flower or fruit to the analysis did not change the coefficients of source and sink strength, but only influenced the baseline hazard. The baseline hazard in the analysis with the position of the flower included was lower than the baseline hazard of the analysis in which the position of the flower was not included. The lower baseline hazard represented the higher survival of fruit on the main shoot, and it was increased for the flowers on the side shoot. Apparently, the average of the hazards for a flower on the main shoot and on the side shoot resulted in the baseline hazard of the analysis without the position of the flower, and hence adding the position on the plant did not improve the simulation.

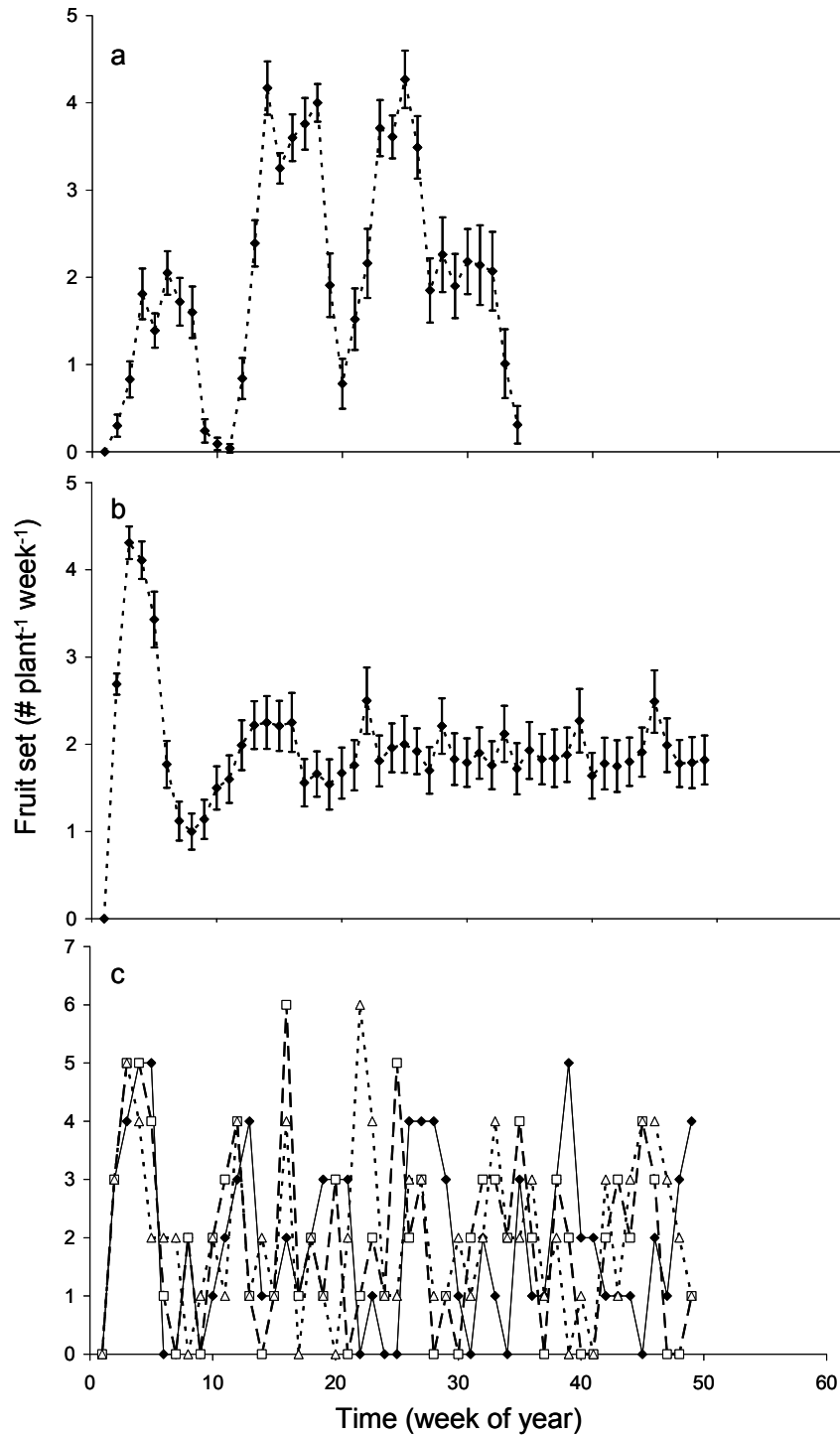


Fig. 7.8 Average fruit set of 100 plants simulated a) under constant leaf area index (2.5) and measured environmental data, b) constant source strength, c) simulated fruit set of three randomly drawn plants from simulation b. Error bars represent standard deviation divided by 4.

We conclude that the model is capable of simulating a realistic fluctuating pattern of fruit set with variation around the simulated pattern. The variation in model output offers the opportunity to conduct ‘virtual experiments’ in order to assess the variability of the output variables under specific circumstances.

Appendix Chapter 7

Model description of the adapted INTKAM model

INTKAM adapted for sweet pepper has shown to simulate accurately the growth of the total crop and the fruit yield of sweet pepper over time (Marcelis *et al.*, 2006). Source strength, dry matter partitioning and organ growth are the same as in the original version of INTKAM. In the adapted version, the calculations and parameters for sink strength are new. The module ‘abortion’ is replaced by the one presented here, and uses the results of survival analysis.

Below a short description is given of essential processes and variables in plant and organ growth and development, including flower and fruit abortion and dry matter partitioning. State and rate variables are defined in Table A7.1, and in Table A7.2 parameters are defined and their values given.

Source strength

Leaf area increase is linearly related to the average daily temperature above a base temperature T_{base} (Marcelis *et al.*, 2006). Interception of photosynthetic active radiation (PAR) is calculated for a multi-layered uniform canopy (Goudriaan and Van Laar, 1994), using Lambert-Beer law (Monsi and Saeki, 1953). Leaf gross photosynthesis is calculated with the biochemical model of Farquhar *et al.* (1980) at five depths in the canopy. Canopy gross photosynthesis is computed from leaf photosynthesis with the Gaussian integration method (Goudriaan and Van Laar, 1994). Light interception and photosynthesis are calculated every hour and integrated over the day to obtain daily values.

Source strength on day t_c is calculated from the canopy gross photosynthesis P_{gross} on day t_c and the maintenance respiration of the plant Maint on day t_c .

$$\text{Source}(t_c) = P_{\text{gross}}(t_c) \cdot 30/44 - \text{Maint}(t_c)$$

$P_{\text{gross}}(t_c)$ is expressed in $\text{g CO}_2 \text{ plant}^{-1} \text{ d}^{-1}$ and is therefore multiplied by 30/44 to convert to $\text{CH}_2\text{O plant}^{-1} \text{ d}^{-1}$. $\text{Maint}(t_c)$ is calculated from the maintenance respiration of the different types of organs and depends on the mass of those organs and on temperature.

$$\begin{aligned} \text{Maint}(t_c) = & (w_l(t_c - 1) \cdot \text{maint}_l + w_{st}(t_c - 1) \cdot \text{maint}_{st} + \\ & w_f(t_c - 1) \cdot \text{maint}_f + w_r(t_c - 1) \cdot \text{maint}_r) \cdot \text{cor}_{T(t_c)} \end{aligned}$$

with $w_l(t_c - 1)$, $w_{st}(t_c - 1)$, $w_f(t_c - 1)$ and $w_r(t_c - 1)$ are the total leaf, stem, fruit and root mass on the previous day respectively, and maint_l , maint_{st} , maint_f and maint_r the maintenance coefficients for leaves, stem, fruits and root, respectively. The correction factor for temperature, $\text{cor}_{T(t_c)}$ is calculated by

$$\text{cor}_{T(t_c)} = Q_{10}^{(T(t_c) - T_{\text{ref}})/10}$$

Q_{10} is the factor by which the maintenance respiration is multiplied with every 10°C increase in temperature, $T(t_c)$ is the average 24h temperature and T_{ref} is the reference temperature for this correction.

Sink strength

The sink strength of a fruit is quantified by its potential fruit growth rate expressed in g CH₂O d⁻¹. Temperature had no direct effect on the sink strength of a fruit: potential fruit mass was the same at 18, 21 and 24°C expressed on temperature sum basis.

The potential fruit growth rate is calculated by a set of equations originating from Chapter 3. The equations from Chapter 3 calculate the potential fruit mass in g fresh mass and the fraction dry matter of the fruit. Both are calculated from the age of the fruit t_{f_i} , expressed in degree-days.

t_{f_i} on day t_c is calculated as the temperature sum above T_{base} from day $z = 1$ (anthesis) till day $z = q$ which coincides with day t_c .

$$t_f(t_c) = \sum_{z=1}^q (T(t_z) - T_{\text{base}})$$

The potential fruit mass, expressed in fresh mass, is calculated with a Richards function.

$$w_{\text{pot}_f, f_i(t_{f_i})}(t_c) = \frac{W_{\text{max}}}{(1 + v \cdot e^{-k \cdot (t_{f_i} - t_m)})^{1/v}}$$

To convert this in potential dry mass, the calculated potential fresh mass of fruit f_i is multiplied by the fraction dry matter of fruit f_i , $f_{dm_{f_i(t_{f_i})}}(t_c)$. The fraction dry matter of fruit f_i at time t_c was transformed to normalise the data.

$$y_{f_i(t_{f_i})} = \arcsin \sqrt{f_{dm_{f_i(t_{f_i})}}(t_c)}$$

$y_{f_i(t_{f_i})}(t_c)$ is given by a Ricker function.

$$y_{f_i(t_{f_i})}(t_c) = c_R - a_R \cdot (t_{f_i} - d_R) \cdot e^{-b_R \cdot (t_{f_i} - d_R)}$$

Fraction dry matter of the fruit is then expressed by the back-transformed $y_{f_i(t_{f_i})}(t_c)$

$$f_{dm_{f_i(t_{f_i})}}(t_c) = \sin^2(y_{f_i(t_{f_i})}(t_c))$$

Potential dry mass is then calculated as

$$w_{\text{pot}_d, f_i(t_{f_i})}(t_c) = w_{\text{pot}_f, f_i(t_{f_i})}(t_c) \cdot f_{dm_{f_i(t_{f_i})}}(t_c)$$

This represents the potential fruit mass expressed in g dry mass at a certain fruit age t_{f_i} . The sink strength is the potential fruit growth rate, which is the derivative of the multiplication of the two functions, obtained using the product rule. This results in a sink strength expressed as g dm fruit⁻¹ °Cd⁻¹. As the source strength is expressed in g CH₂O plant⁻¹ d⁻¹, the sink strength of the fruit has to be converted. The term $Asrqt_f$ is used to convert from g dm to g CH₂O, and the term $(T(t_c) - T_{base})$ is used to convert from degree-days to days. Calculation of derivatives involves the use of the chain rule, product rule and quotient rule.

$$Sink_{f_i(t_{f_i})}(t_c) = Asrqt_f \cdot (T_{t_c} - T_{base}) \cdot (w_{pot_f, f_i(t_{f_i})}(t_c)) \cdot \frac{d fdm_{f_i(t_{f_i})}(t_c)}{dt} + \frac{d w_{pot_f, f_i(t_{f_i})}(t_c)}{dt} \cdot fdm_{f_i(t_{f_i})}(t_c)$$

The sink strength for fruit f_i is calculated daily between anthesis and abortion, or harvest. A fruit is removed from the simulation when its age t_{f_i} is equal to or larger than the age of harvest or when it aborts.

On an arbitrary simulation day, fruits of different ages and hence with different sink strengths are present. The total sink strength on day t_c , $Sink_{TF}(t_c)$ is calculated from the sink strength of all $n(t_c)$ fruits present on day t_c .

$$Sink_{TF}(t_c) = \sum_{i=1}^{n(t_c)} Sink_{f_i(t_{f_i})}(t_c)$$

The vegetative sink strength increased with increasing temperature. The slope s_T was determined in the temperature experiment described in Chapters 3 and 6. $Sink_{V,0}$ is the vegetative sink strength at the reference temperature of 20°C. $Sink_V(t_c)$ is expressed in CH₂O plant⁻¹ d⁻¹.

$$Sink_V(t_c) = s_T \cdot (T(t_c) - 20) + Sink_{V,0}$$

From the total fruit sink strength on day t_c and the vegetative sink strength on day t_c the total sink strength on day t_c is calculated.

$$Sink_{TOT}(t_c) = Sink_V(t_c) + Sink_{TF}(t_c)$$

The total sink strength and the source strength are now known. These are the basis for the calculation of flower and fruit abortion, dry matter partitioning and growth of the individual organs.

Abortion

For each fruit in the critical period for abortion, which ranged from 50°Cd before anthesis till 150°Cd after anthesis, the hazard rate was calculated daily. The fruit age in this calculation is represented by t_{f_i} , where

$$t_{f_i'} = t_{f_i} + 50$$

Hence, the fruit age $t_{f_i'}$ used in the calculation of the abortion rate is 50°Cd higher than the fruit age t_{f_i} used in the other calculations for the fruit.

The baseline hazard rate $h_{0,f_i(t_{f_i'})}(t_c)$ of fruit f_i , or probability of abortion per degree-day in absence of the influencing factors, is calculated by the first derivative of a Gompertz function (Fig. 1A of Chapter 7).

$$h_{0,f_i(t_{f_i'})}(t_c) = h_{\max} \cdot k_h \cdot e^{-e^{-k_h \cdot (t_{f_i'} - t_{m,h})}} * e^{-k_h \cdot (t_{f_i'} - t_{m,h})}$$

The probability of abortion per day, taking into account the effect of source and sink strength, and, if desired, position (POS) on the plant, as well as the conversion from degree-days to days, is given below.

$$h_{f_i(t_{f_i'})}(t_c) = h_{0,f_i(t_{f_i'})}(t_c) \cdot (T_{t_c} - T_{base}) \cdot e^{So_1 \cdot Source(t_c - 1:t_c) + So_2 \cdot Source^2(t_c - 1:t_c) + So_3 \cdot Source^3(t_c - 1:t_c) + Si_2 \cdot Sink_{TOT}^2(t_c - 9:t_c) + Si_3 \cdot Sink_{TOT}^3(t_c - 9:t_c) + p \cdot Pos}$$

$Source(t_c - 1:t_c)$ and $Sink_{TOT}(t_c - 9:t_c)$ are here the source strength the present and previous day and sink strength averaged over the present and 9 previous days, respectively. The term $(T_{t_c} - T_{base})$ is used to convert to probability per day.

After this probability per day, hazard rate, has been calculated, a random number, RAND, is drawn from a uniform distribution between 0 and 1. This random number is compared to the hazard rate expressed in days.

If $h_{f_i(t_{f_i'})}(t_c) \geq RAND$, the flower or fruit would abort, else the flower or fruit does not abort on this day. However, for flowers and fruits which are still in the critical period for abortion, it might be that they abort on one of the coming days.

Dry matter partitioning and organ growth

The assimilates are divided between fruits and vegetative organs based on the concept of sink strengths (Marcelis, 1996). The amount of assimilates partitioned to a (group of) organs equals the total amount of available assimilates multiplied by the organ's share in the total sink strength.

The assimilates partitioned into the vegetative part on day t_c $Ass_v(t_c)$ is given by

$$Ass_v(t_c) = Source(t_c) \cdot \frac{Sink_v(t_c)}{Sink_{TOT}(t_c)}$$

The assimilates partitioned into the fruits on day t_c is given by

$$Ass_f(t_c) = Source(t_c) \cdot \frac{Sink_{TF}(t_c)}{Sink_{TOT}(t_c)}$$

These equations can be rearranged to

$$Ass_v(t_c) = \frac{Source(t_c)}{Sink_{TOT}(t_c)} \cdot Sink_v(t_c)$$

$$Ass_f(t_c) = \frac{Source(t_c)}{Sink_{TOT}(t_c)} \cdot Sink_{TF}(t_c)$$

This shows that the growth rate of the vegetative parts and the fruits is proportional to the source-sink ratio given by $\frac{Source(t_c)}{Sink_{TOT}(t_c)}$. If the source-sink ratio is larger than 1 (higher supply of assimilates than demand for assimilates), all organs grow potentially and the excess assimilates are added to the source strength of the next day.

Next, the assimilates are converted into dry mass. First the total vegetative growth is calculated. Conversion of assimilates (g CH₂O) to dry mass is done with a factor representing the assimilate requirement per gram dry mass $Asrqt_v$.

$$Growth_v(t_c) = Ass_v(t_c) / Asrqt_v$$

From this total vegetative growth rate, the growth of the roots is calculated

$$Growth_r(t_c) = R_r \cdot Growth_v(t_c)$$

For the division between stem and leaves an allometric relation based on the total dry matter in the shoot is used. The total dry matter in the shoot (stem and leaves) is calculated as:

$$w_{shoot}(t_c) = w_l(t_c - 1) + w_{st}(t_c - 1) + (Growth_v(t_c) - Growth_r(t_c))$$

The mass of the leaves is calculated from the shoot dry matter.

$$w_l(t_c) = e^{a+b \cdot \ln(w_{shoot}(t_c))}$$

The growth rate of the leaves is thus quantified by

$$Growth_l(t_c) = w_l(t_c) - w_l(t_c - 1)$$

And the growth of the stem by

$$Growth_{st}(t_c) = Growth_v(t_c) - Growth_r(t_c) - Growth_l(t_c)$$

Total dry matter in the roots is then calculated by

$$w_r(t_c) = w_r(t_c - 1) + Growth_r(t_c)$$

And the total dry matter in the stem by

$$w_{st}(t_c) = w_{st}(t_c - 1) + Growth_{st}(t_c)$$

The assimilates partitioned into the fruits are divided among the individual fruits based on their share in the total fruit sink strength $Sink_{TF}(t_c)$.

$$Ass_{f_i}(t_c) = Ass_f(t_c) \cdot \frac{Sink_{f_i(t_{f_i})}(t_c)}{Sink_{TF}(t_c)} \text{ which is equal to}$$

$$Ass_{f_i}(t_c) = Source(t_c) \cdot \frac{Sink_{f_i(t_{f_i})}(t_c)}{Sink_{TOT}(t_c)} \text{ or } Ass_{f_i}(t_c) = Sink_{f_i(t_{f_i})}(t_c) \cdot \frac{Source(t_c)}{Sink_{TOT}(t_c)}. \text{ Hence, the}$$

real growth rate of a fruit, expressed in $g \text{ CH}_2\text{O fruit}^{-1} \text{ d}^{-1}$, is proportional to the source-sink ratio.

The new mass of a fruit is the assimilates converted to dry mass added to the mass of the fruit.

$$w_{f_i}(t_c) = w_{f_i}(t_c - 1) + Ass_{f_i}(t_c) / Asrqt_f$$

Total plant dry matter is then calculated as

$$w_p(t_c) = w_l(t_c) + w_{st}(t_c) + w_r(t_c) + \sum_{i=1}^{n(t_c)} w_{f_i}(t_c)$$

Table A7.1 State and rate variables with their units.

Variable	Unit	explanation
t_c	d	Time expressed in calendar day
t_f	°Cd	Age of fruit i
t_f	°Cd	Age of fruit used in abortion function ($t_f + 50$)
$T(t_c)$	°C	Average 24h temperature on day t_c
$Source(t_c)$	g CH ₂ O plant ⁻¹ d ⁻¹	Source strength on day t_c
$P_{gross}(t_c)$	g CO ₂ plant ⁻¹ d ⁻¹	Gross assimilation on day t_c
$Maint(t_c)$	g CH ₂ O plant ⁻¹ d ⁻¹	Maintenance respiration on day t_c
$w_l(t_c)$	g dm	Leaf mass on day t_c
$w_{st}(t_c)$	g dm	Stem mass on day t_c
$w_r(t_c)$	g dm	Root mass on day t_c
$w_f(t_c)$	g dm	Total mass of fruits on day t_c
$w_{f_i}(t_c)$	g dm	Mass of fruit i on day t_c
$w_{shoot}(t_c)$	g dm	Shoot mass (leaves and stem) on day t_c
$w_p(t_c)$	g dm	Plant mass on day t_c
$cor_{T(t_c)}$	-	Correction factor for temperature on maintenance respiration
$w_{pot_{f_i}(t_f)}(t_c)$	g fm	Potential fresh mass of fruit i with age t_{f_i} on day t_c
$f_{dm_{f_i}(t_f)}(t_c)$	-	Fraction dry matter of fruit i with age t_{f_i} on day t_c
$w_{pot_{d_{f_i}(t_f)}(t_c)}$	g dm	Potential dry mass of fruit i with age t_{f_i} on day t_c
$Sink_{f_i(t_f)}(t_c)$	g CH ₂ O fruit ⁻¹ d ⁻¹	Sink strength of fruit i with age t_{f_i} on day t_c

Table A7.1 continued

Variable	Unit	explanation
$Sink_{IF}(t_c)$	g CH ₂ O plant ⁻¹ d ⁻¹	Total fruit sink strength on day t_c
$Sink_V(t_c)$	g CH ₂ O plant ⁻¹ d ⁻¹	Vegetative sink strength on day t_c
$Sink_{TOT}(t_c)$	g CH ₂ O plant ⁻¹ d ⁻¹	Total sink strength on day t_c
$h_{0,f_i}(t_c)$	°Cd ⁻¹	Baseline hazard rate for fruit i with age t_f
$h_{f_i}(t_c)$	d ⁻¹	Hazard rate for fruit i with age t_{f_i} , accounting for factors $X_{j(i)}$
$Source(t_c - 1 : t_c)$	g CH ₂ O plant ⁻¹ d ⁻¹	Source strength averaged over previous and present day
$Sink_{TOT}(t_c - 9 : t_c)$	g CH ₂ O plant ⁻¹ d ⁻¹	Total sink strength averaged over previous nine days and present day
Pos	-	Position of flower or fruit on plant, 0 main branch, 1 side branch
$RAND$	-	Random number drawn from uniform distribution between 0 and 1
$Ass_V(t_c)$	g CH ₂ O d ⁻¹	Assimilates partitioned into the vegetative parts on day t_c
$Ass_f(t_c)$	g CH ₂ O d ⁻¹	Assimilates partitioned into the fruits on day t_c
$Ass_{f_i}(t_c)$	g CH ₂ O d ⁻¹	Assimilates partitioned into fruit i with age t_{f_i} on day t_c
$Growth_V(t_c)$	g dm d ⁻¹	Growth rate of vegetative part on day t_c
$Growth_r(t_c)$	g dm d ⁻¹	Growth rate of roots on day t_c
$Growth_l(t_c)$	g dm d ⁻¹	Growth rate of leaves on day t_c
$Growth_{st}(t_c)$	g dm d ⁻¹	Growth rate of stem on day t_c

Table A7.2 Parameter and their values.

Parameter	value	Unit*	Explanation
Q10	2	-	Multiplication factor for maintenance respiration when temperature increase 10°C
T _{ref}	25	°C	Reference temperature for maintenance respiration
maint _l	0.025	g CH ₂ O g dm ⁻¹	Sugars mass needed to maintain one gram of leaf weight
maint _{st}	0.025	g CH ₂ O g dm ⁻¹	Sugars mass needed to maintain one gram of stem weight
maint _f	0.010	g CH ₂ O g dm ⁻¹	Sugars mass needed to maintain one gram of fruit weight
maint _r	0.025	g CH ₂ O g dm ⁻¹	Sugars mass needed to maintain one gram of root weight
T _{base}	10	°C	Base temperature for development
w _{max}	225	g fm	Potential fresh mass of a fruit
k	0.01115	°Cd ⁻¹	Rate parameter of the Richards function
t _m	293.36	°Cd	Time of maximum weight increase
v	0.30374	-	Shape parameter of the Richards function
c _R	0.3383	-	Parameter Ricker function
a _R	-0.00084	°Cd ⁻¹	Parameter Ricker function
b _R	0.00377	°Cd ⁻¹	Parameter Ricker function
d _R	78.778	°Cd	Parameter Ricker function
s _r	0.16	g CH ₂ O plant ⁻¹ °Cd ⁻¹	Increase of vegetative sink strength with 1°C in temperature
Sink _{V,0}	20.2	g CH ₂ O plant ⁻¹ d ⁻¹	Vegetative sink strength at 20°C
h _{max}	5.1772 / 4.44 [#]	-	Cumulative baseline hazard at infinity
k _h	-0.015305	°Cd ⁻¹	Rate parameter in Gompertz function for hazard rate
t _{m,h}	119.8345	°Cd	Time of maximum hazard (since 50°Cd before anthesis)
So ₁	-1.25	(g CH ₂ O plant ⁻¹ d ⁻¹) ⁻¹	Parameter for effect source strength in hazard rate
So ₂	0.1607	(g CH ₂ O plant ⁻¹ d ⁻¹) ⁻²	Parameter for effect source strength in hazard rate
So ₃	-0.009	(g CH ₂ O plant ⁻¹ d ⁻¹) ⁻³	Parameter for effect source strength in hazard rate
Si ₂	0.052	(g CH ₂ O plant ⁻¹ d ⁻¹) ⁻²	Parameter for effect sink strength in hazard rate
Si ₃	-0.0026	(g CH ₂ O plant ⁻¹ d ⁻¹) ⁻³	Parameter for effect sink strength in hazard rate
p	0.3157	-	Parameter for flower of fruit positioned on side branch
R _r	0.1	-	Ratio of root weight in total vegetative weight
a	-0.394	-	Intercept in allometric relation between leaf weight and shoot weight
b	0.896	-	Slope in allometric relation between leaf weight and shoot weight
Asrq _{lv}	1.295	g CH ₂ O g dm ⁻¹	Assimilate requirement per gram vegetative tissue
Asrq _f	1.35	g CH ₂ O g dm ⁻¹	Assimilate requirement per gram fruit

* dm = dry mass, fm = fresh mass

first value for simulation in which position of the flower or fruit is not taken into account, second value for simulation in which position of the flower or fruit is taken into account

Chapter 8

General discussion

The aim of this thesis has been to introduce stochasticity into a dynamic simulation model. In this way, the model output differs per simulation run, and the average simulation output and the variation around the average is obtained. Flower and fruit abortion in sweet pepper was chosen as case study, which shows strong variation over time as well as between plants. I expected an accurate simulation of the average fruit set pattern, as well as the variation around the mean. Survival analysis has been used as the method to derive the function which introduces stochasticity into the model. Survival analysis of flower and fruit abortion data yielded an abortion rate depending on its influencing factors. First, I discuss whether survival analysis is the appropriate method to analyse flower and fruit abortion data and how universal the obtained function is. Secondly, sink strength and fruit set are examined. Finally, I focus on the stochastic simulation, and on which other methods could have been used and the prospects of stochastic dynamic simulation models.

Survival analysis

Is survival analysis the appropriate method to analyse flower and fruit abortion data?

Survival analysis has been applied to establish a relation between the factors influencing abortion and the occurrence rate of fruit abortion. I used the Cox model to parameterize this relation. The Cox method is capable of analyzing survival times with time-dependent covariates, dependence between the data can be taken into account and there is no need to predefine the shape of the baseline hazard.

An important question is whether it was necessary to incorporate the timing of abortion in the analysis. Ultimately, the amount of aborting flowers and fruits is important, not when the flowers abort. However, Marcelis and Baan Hofman – Eijer (1997) showed that the presence of flowers or young fruits, even if these aborted after some days, influenced the probability of abortion of the next flowers. In this respect, analyzing the timing of abortion and incorporating the presence of aborted fruit in the covariates, is the correct approach.

In case the timing of abortion is omitted, the analysis is reduced to the question: does a flower or fruit abort or not? This abortion probability has to be related to explanatory variables. Such an analysis is done with a generalised linear (mixed) model with a binary response variable. In such a case, an explanatory variable like source strength has to be defined differently, like the average over the first five days after anthesis (Wubs *et al.*, 2008). This is a disadvantage, because a change in source strength or temperature after the first five days after flowering can also induce abortion (Marcelis *et al.*, 2004). Taking a longer period over which such a variable is averaged, e.g. 10 days, can imply that the explanatory variable is not relevant any more for flowers that have aborted within 10 days

after anthesis. Another disadvantage is that this method cannot deal with individuals which have not yet experienced the event (censored observations). For a generalized linear (mixed) model these censored observations have to be critically inspected. If the individuals with censored survival times are still in the critical period for abortion, they cannot be used in the analysis. Hence, from the same data set, less observations can be included using a generalized linear model with binary response variable than when using survival analysis.

A more elaborate binary analysis of survival data is the use of interval counts (Egli and Schmid, 2001; Kleinbaum and Klein, 2005). The mortality rate is defined per interval. Censored observations can be used in this analysis: individuals censored at time t are included in the calculations for mortality rate at interval between $t-1$ and t , and omitted from the later intervals. Egli and Schmid (2001) applied this method to survival data of leaf cohorts. The method of analysis allows the use of time dependent covariates. Treatment effects can be tested against their appropriate error terms, and any survivorship function can be fitted (Egli and Schmid, 2001). However, survival analysis also has these properties, and as such, the proposed method has no advantages over the method of survival analysis applied presently.

Another possible survival model is the accelerated failure time model (Fox, 1993). In this model, the covariates are multiplicative with respect to survival time, while in the Cox model the covariates are multiplicative with respect to the hazard (Kleinbaum and Klein, 2005). The choice between the two models should be based on biological assumptions (Fox, 1993). The baseline hazard is predefined and time-dependent covariates are difficult to implement in accelerated failure time models. For the modelling purpose, it was important that the time-dependent covariates influence the probability of abortion rate per day rather than the timing of abortion. Therefore, in this case the Cox model was preferred above the accelerated failure time model.

It can be concluded that survival analysis with the Cox model was the appropriate method to assess the influence of source and sink strength on the abortion rate.

Dependency of the data

One assumption in all above-mentioned analyses, as with all regression models, is independence of the data. Fruits growing on the same plant have in common that they are dependent on the specific circumstances of this plant, and this has been accounted for by the use of a frailty term (Therneau and Grambsch, 2000). A frailty term can be compared to a random factor in a mixed model. However, the dependency in the abortion data of sweet pepper flowers and fruits is more complex: the survival of one individual influences the survival of the next individuals. The survival time of fruits is the response variable and indirectly part of the explanatory variable (for other individuals) at the same time. The explanatory variables sink strength and age classes of the fruits account for this dependency, but their accuracy relies on the accuracy of the survival times. To overcome this problem of dependency between the survival times, experiments have to be performed in which the survival time of one flower is studied in presence of one or more other fruit. These other fruit have to be of different ages, and the experiment should be repeated at different source strengths. This makes obtaining flower and fruit abortion data cumbersome. The present data sets had their limitations, but made sure that all naturally occurring combinations of source and sink strength were present.

Dependency does not only exist in the survival times, but also in the covariates: there was a positive correlation between source and sink strength. When a crop is planted in December, which is the usual planting period, source strength increases in the period up to June due to the increase in leaf area per plant and the increase in incoming radiation. In this period, fruit set and consequently sink strength also increases due to increase in source strength. In the analysed density experiment, source and sink strength were more or less independent in July and August. An experiment in which different starting dates are applied (e.g. December, March and May) or a constant light level is achieved by supplemental lighting and screening might result in lower dependency of the sink strength on the source strength. It should be noted that it is impossible to achieve complete independence of source and sink strength.

Covariates

Whereas the sink strength has been calculated separately for each plant, the source strength used in the survival analysis is assumed to be the same for every plant of a treatment. However, inter-plant variation in source strength likely exists. First, the leaf area and hence the amount of intercepted light varies from plant to plant. This has most impact when the canopy is not yet closed and results in different source strengths. Secondly, the simulated source strength used in the survival analysis originated from one simulation run, with a particular pattern of set fruit. However, a different fruit set pattern would have lead to slightly different source strengths. The first mentioned factor causes larger variation than the second one, especially in the first few months of cultivation, but it is also more difficult to quantify this source of variation. Leaf area is determined during the destructive measurements and therefore an average value over time is known, not the leaf area development of a specific plant. Although plant specific measurements can be obtained by measuring leaf area non-destructively (method from De Swart *et al.*, 2004), it adds an extra work load on the already intensive observations of flowering, abortion and fruit harvest.

I have assumed that not the daily values of assimilate supply and demand, but averages over several days were regulating abortion. This is in accordance with findings in soybean (Egli and Bruening, 2006a) and the tropical shrub *Melastoma malabathricum* (Kamoi *et al.*, 2008). However, two weeks of assimilate deprivation which soybean pods can withstand before abortion (Egli, 2010) is much longer than the period of assimilate deprivation sweet peppers can withstand. Marcelis *et al.* (2004) report high abortion (up to 100%) after seven days of shading. Kamoi *et al.* (2008) reported that abortion rates of reproductive organs of *M. malabathricum* correlated best to (a measure of) source-sink ratio when this measure was averaged over 5 days.

How universal is the estimated survival function?

The abortion function has, in the first place, been developed for large block-type sweet peppers. The sink strength function is determined with the cultivar ‘Mazurka’ (Chapter 3), but the survival analysis and its calibration in the simulation model were based on the cultivar ‘Red Spirit’. However, discrepancies between simulated and observed fruit set patterns were not cultivar-related, but due to the absence of certain combinations of source and sink strength in the analysed data. Validation with the cultivar ‘Mazurka’ (1st validation data set) are good. Validation with the cultivars ‘Spider’ and ‘Orange Glory’ are reasonable, but more cultivar specific parameters might have given better results. ‘Spider’ is known as a cultivar in which fruits are less vulnerable for abortion. This can be due to a

lower baseline hazard, so a difference in the survival function, but can also be due to a lower vegetative sink strength.

The timing of abortion in flowers and fruits is nearly similar in different cultivars (Fig. 4.2). Hence, the shape of the baseline abortion rate, which is related to the Kaplan-Meijer survival function, are similar for different cultivars, although the positioning of the period of high abortion rate might vary some days. However, the baseline hazard rate might be different. The different source-sink ratio thresholds for fruit set (Chapter 5) indicate a lower baseline hazard for cultivars with small-sized fruits, or a larger effect of source-sink ratio on abortion rate, or, most likely, a combination of these two.

Temperature was not included as a separate covariate in the survival analysis. Temperatures between 18 and 24°C were assumed to influence abortion only via source and sink strength. However, it is known that pollen development is hampered under very high temperatures (33°C, Erickson and Markhart, 2002; 32/26°C day/night, Aloni *et al.*, 2001) or low night temperatures (10°C, Mercado *et al.*, 1997; 12°C, Pressman *et al.*, 1998). The obtained survival function is not sufficient for very low or very high temperatures, and an additional effect of temperature should be modelled. Additional experiments should be conducted for the quantification of this effect.

Sink strength

Sink strength is one of the key variables in many simulation models, and influences dry matter partitioning and fruit abortion (Marcelis, 1996). Sink strength itself is a term which has evoked a lot of discussion in the past (Farrar, 1993), and it still does. It is often quantified as the growth rate of a fruit, sometimes the actual growth rate (González-Real *et al.*, 2009), sometimes the potential growth rate (Grossman and DeJong, 1995). In other cases, it has been merely used to indicate changes in the number of fruits (sinks), without actually quantifying sink strength (e.g. Alkio *et al.*, 2003; Egli and Bruening, 2003; Valantin *et al.*, 1998). Many authors agree that a delay between fruit set and the fruit's maximum competitive strength causes the fluctuations in fruit set within a plant (e.g. Buwalda *et al.*, 2006). The sink strengths used in Chapters 5, 6, and 7 were derived from the fruit growth curves fitted in Chapter 3.

Sink strength, in this thesis, has been quantified by the potential growth rate of the fruit. It depends entirely on the assimilate requirement of the fruit, although it is known that dominance due to hormonal flows is a factor as well (Bangerth, 1989). However, fruit growth is easier to measure than hormone levels within a fruit and the export of hormones from the fruit. The vegetative sink strength has been derived from the calculated generative sink strength, the simulated source strength and the observed dry matter partitioning. This has been assumed constant in time, only to increase with increasing temperature and to differ between cultivars. This is a rather strong assumption, and investigation of the vegetative sink strength deserves more attention.

An important issue regarding the determination of the sink strength is whether the growth of the measured fruits was really potential. Plants in the temperature experiment, which were used for determination of the fruit sink strength, were growing in the pots filled with peat instead of growing in rockwool slabs, and were cultivated in a glasshouse with low transmission. The experiment was repeated in autumn, and there fruit size of fruit growing without competition decreased with higher temperatures (Lee, 2008). As this was

not observed in spring when radiation levels were higher, the fruit size in autumn must have been source-limited, and, under the circumstances of the cultivation, potential sink size was reached in spring. However, ideal experiments to obtain the sink strength should be performed under levels of high radiation and high levels of CO₂.

A second issue is whether incorrectly estimated sink strength matters. The ‘real’ potential fruit growth is not known. A clue can be derived from the source-sink ratios. If the estimated sink strength is low, it would result in relatively high source-sink ratios. Source-sink ratios which exceed one for a long period, or are much higher than one, are unlikely in sweet pepper: it indicates that growth is sink-limited rather than source-limited. However, a large number of potential sinks (flowers) is produced, but they abort due to low assimilate availability. The source-sink ratios in the density experiment of Chapter 6 were most of the time lower than one (Fig. 8.1).

Regarding the consequences of misestimated sink strength it should be noticed that as long as the same sink strength is used in the simulation model and in the estimation of the abortion function, it makes no difference. In that case, the parameters of the abortion function are then based on the too low values for sink strength, but they also are used in combination with the too low values of the sink strength. However, the applicability of the function in other situations is limited, as other environmental circumstances can lead to different sink strengths.

Potential fruit size

Even though the fruits were grown with little or no competition, fruit size varied. Sadras and Denison (2009) state that number and potential size of grain crops is determined by the mother plant before fertilization. An analysis by Gambín and Borrás (2009) on different crops (mainly grain and bean crops) indicated that final seed weight was related to the crop growth rate per seed during the seed set period.

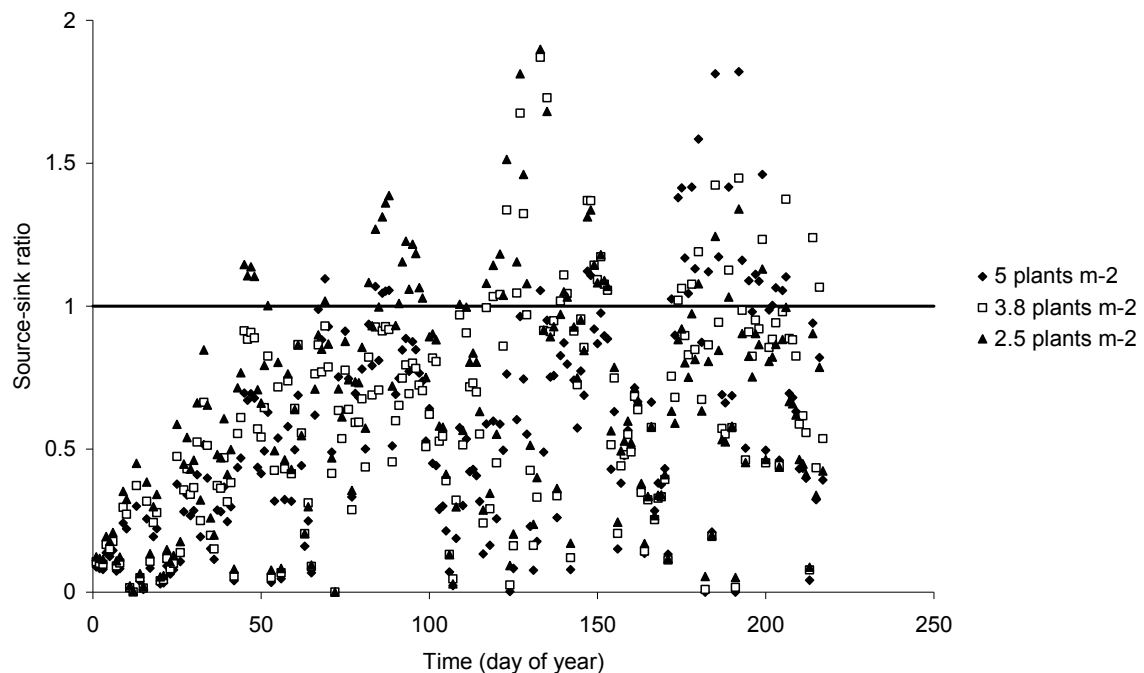


Fig. 8.1 Average daily source-sink ratios in the density experiment of Chapter 6.

The size of a fruit depends on cell number as well as cell size (Gillaspy *et al.*, 1993). In *Lycopersicum pimpinellifolium* mutants, cell number was strongly related to fruit size. Cell division, determining cell number, mainly occurs up to 7 to 10 days after fertilisation (Bohner and Bangerth, 1988). In all these cases, fruit or seed size is determined during the early development of the fruit or seed. Assuming similar principles in sweet pepper fruit, differences in final fruit size might be due to different number of cells, caused by pre-anthesis environmental effects on the development of the bud. If the number of fruits per plant during the development of the buds differed among the plant, this has affected the development of the bud: a higher fruit load decreases bud size and the number of cells in developing buds of tomatoes (Baldet *et al.*, 2006). This may imply that sink strength is dependent of the circumstances during early development of the fruit.

Temperature had no influence on the potential fruit dry weight of sweet pepper fruits in the range of temperatures used in the experiment. This corresponds to observations in tomato (Heuvelink and Marcelis, 1989), but in cucumber, the potential dry weight of fruits decreased with lower temperature (Marcelis and Baan Hofman-Eijer, 1993). The size of the sweet pepper fruits grown at the highest temperature (24°C) seems to be slightly smaller than the size of fruit grown at 18 and 21°C, although the difference was not significant. Most fruits at the highest temperature suffered from blossom-end rot (BER) and were omitted from the analysis. BER is correlated with high initial fruit growth rate (Marcelis and Ho, 1999). So, the fruits at 24°C from which a reliable fruit growth curve could be obtained were somewhat slower growing fruits.

Temperature can affect cell number and cell size. In cucumber, cell size increased with increasing temperature, while cell number was not affected (Marcelis and Baan Hofman-Eijer, 1993). In tomato, however, both cell number and cell volume were affected: cell volume was higher under higher temperature (25°C /25°C day/night), but cell number was higher under lower temperatures (20°C /20°C day/night) (Bertin, 2005). As a result, fruit fresh weight did not differ between the two temperatures (Bertin, 2005). As with tomato, the sweet pepper fruit size did not differ between the temperatures, both cell volume and cell number are likely to be affected. Cultivar differences in fruit sink strength can be caused by differences in the number of cells, as was found in sweet cherry fruits (Olmstead *et al.*, 2007).

Fruit set

Fruit set was the primary variable of interest in this thesis. It is the complement of fruit abortion: if a fruit doesn't abort, it is considered as set. This was the definition used in this thesis. However, the definition of fruit set varies among researchers. A fruit is often considered as set when it has reached a certain size (13 mm), but is still susceptible for abortion. In such a definition, a set fruit can abort.

Observation of fruit abortion and fruit set is a laborious task, which requires frequent observations on several plants. It can not be avoided that plants are touched or otherwise disturbed in their growth. It is often noticed that the plants which are used for observations of fruit set after some time behave differently from the other plants in the greenhouse, e.g. shorter plants, asynchronous fruit set compared to other plants in the greenhouse (pers. comm., Arie de Gelder, Steven Driever). It should therefore be kept in

mind that the presented fruit set patterns are not necessarily representative for the whole greenhouse.

Building stochasticity into dynamic simulation models

Stochasticity in the model simulations was obtained by implementing the abortion function derived with survival analysis into the INTKAM model. A probability rate per day was calculated for each flower and fruit during its critical period for abortion. The probability rate differed per day and per fruit, depending on fruit age and the source and sink strength. A similar method was used by Lieth *et al.* (1986) to simulate boll abortion in cotton, but they did not estimate the abortion equation with survival analysis. Variation in simulation output is in these cases an intrinsic part of the simulation model, i.e. it is generated during the simulations, and does not result from variation of input data or parameter values. A similar approach is the use of the results of a binomial regression, where a probability is calculated depending on certain factors. A simplified form is the use of a constant probability, which does not depend on other factors. In all these three cases, the (calculated) probability (rate) is compared to a random number drawn from an uniform distribution between 0 and 1, from which it is decided whether the event happens or not. These methods are often used in individual-based simulation methods of population dynamics (e.g. Meyer *et al.*, 2009b; Vos and Hemerik, 2003).

Variation in processes can also be simulated when a certain distribution of variation around mean parameter values is assumed. Parameter values can vary between different individuals in the same simulation or between simulation runs. Variation in rates is common, like the rate of truss formation in tomato (Pearson *et al.*, 1996), and the spear growth rate in asparagus (Graefe *et al.*, 2009). The first assumed a normal distribution of truss formation rate, where the mean depended on the temperature and the standard deviation was constant. In asparagus, a normal distribution was also assumed for variation in spear growth rate between bud clusters and between the buds in a cluster (Graefe *et al.*, 2009). Variation of leaf area development in sugar beet was simulated by Chalabi *et al.* (1986) with stochastic differential equations. Further, frequency distributions for the number of organs can be assumed, like the number of flower buds per branch (Agostini *et al.*, 1999), or the number of potential spears in asparagus (Graefe *et al.*, 2009).

Variation in the output can also be obtained by variation in initial conditions or weather data. Using a range of weather data, obtained with a stochastic weather generator, is often used to assess crop sensitivity to variations in weather (e.g. Semenov *et al.*, 2009). It mostly reflects weather conditions in different years, but can also represent variation on a spatial scale. In a greenhouse, it is likely that there is spatial variation in radiation and temperature levels. This variation contributes to the variation in fruit set patterns between the plants. If the spatial variability in environmental data is known, simulating with different environmental data, each representing a certain area in the greenhouse, can be a suitable option as well. Bojacá *et al.* (2009) measured spatial variation in temperature on a 20*22 m grid, interpolated the data to obtain values for each square meter and used the resulting temperature data in simulations of a tomato crop. This resulted in a difference in yield of 3.3 kg m⁻² (tomato fresh fruit weight) between the warmest and coolest square meter. The results were not verified against measured data, but show that spatial variability in temperature in theory has considerable effect on spatial variability in yield.

Last method to obtain variation is to assume a certain distribution around the simulated mean value (method of Benjamin *et al.*, 1999). Variation as an intrinsic part of model simulations has some advantages over applying variation to an output value. The values of output variables are likely to be correlated, and adding variability to one output variable and not to the other can lead to unrealistic combinations of output values. The other advantage of variation as an intrinsic part of the model is that the variation responds to the environmental and physiological circumstances.

In theory, numerous options for stochastic simulated processes are possible. Which process to simulate stochastically depends on importance of the process in crop growth and development, and the method to use depends on the process. However, introducing variation in the simulation of a large number of processes in the same model is not advisable. Too many stochastic processes might lead to fuzzy outcomes, and for proper simulation, the correlation between the variation of the different processes should be known as well. It is also not necessary to simulate a lot of process with variation, as variation in one process leads to variation in several output variables.

Applications

Stochasticity is, up till now, scarcely used in crop models. However, crop simulation models with a stochastic component can serve potential useful purposes. When conducting an experiment, several replicates per treatment are done because not only the mean, but also the variation around the mean is of interest. This variation is needed to statistically compare the treatments. This approach is also valid for ‘experiments’ with a stochastic simulation model. An experiment, in this case, is a change in e.g. a parameter value or crop management. If for example the resulting mean crop weight is 20, this has a different impact when the variation around this value is between 15 and 25, than when it varies between 10 and 30. In commercial settings, stochastic simulation models could be used for risk assessment, as is often done with models in pest management (e.g. Van Mourik *et al.*, 2008; Ezanno and Lesnoff, 2009). Related to this is the use of stochastic models in decision support systems. In agricultural products, estimating the quantity as well as the quality and the variation therein in advance is desirable for stakeholders in the production chain. I expect that the increasing demand for accurate predictions of yield and quality will promote the use of stochastic mechanistic simulation models.

Conclusion

The aim of the project was to introduce stochasticity into the model for the simulation of fruit set. I hypothesised that this would lead to accurate simulation of fruit set and the variation therein. The survival analysis served as a proper tool for estimating the abortion function. In general, the model was able to simulate the observed fruit set pattern. Plant-to-plant variation in model output offers the opportunity to conduct ‘virtual experiments’ in order to assess the variability of the output variables under specific circumstances. Adding a stochastic component to simulation models improves their applicability in decision support systems and risk assessment.

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Summary

Crop models are widely used in research and education, and are increasingly used in greenhouse industry as well. Most crop simulation models are deterministic: with specific parameter values and environmental conditions the model always produces the same output. However, in reality, variation in growth and development exists between plants within the same crop. Simulation of crop growth with variation is therefore more realistic. The objective of this thesis was to introduce a stochastic component into a dynamic crop growth simulation model. In this way, the output of the model differs for each simulation run, and the mean and the variation around it can be obtained.

In the Netherlands sweet pepper plants are cultivated nearly all year round in greenhouses. During the season, large fluctuations in fruit yield can be observed. These yield fluctuations are caused by alternating periods of high and low fruit abortion. Competition between developing flowers and fruits is a major cause of abortion: fast growing fruits cause the abortion of flowers and young fruits. Besides variation in time, there is also variation in fruit abortion between the plants: the number and positions of the set fruit differ between the plants. This variability of fruit abortion between plants makes fruit abortion in sweet pepper a good case study for the introduction of stochasticity into a dynamic simulation model. Survival analysis is the method used to obtain a function which introduces stochasticity into the model. It analyses the timing of the abortion events and relates the abortion rate to influencing factors. The hypothesis was that this would lead to accurate simulation of the observed fruit set patterns and the plant-to-plant variation therein.

In Chapter 2, the stages susceptible to fruit abortion and the factors influencing fruit abortion in sweet pepper are reviewed on the basis of literature. Stages susceptible to abortion are very young buds (< 2.5 mm), buds close to anthesis, and flowers and fruits up to 14 days after anthesis. Source strength and sink strength are two important factors influencing abortion. Source strength is the supply of assimilates originating from photosynthesis. If the source strength is higher, fewer fruits abort. Source strength is increased by increasing radiation and CO_2 levels, and by decreasing planting density. Water and nutrient stress can decrease the source strength. A higher temperature increases source strength, but it decreases at temperatures above 30°C . Sink strength is the demand for assimilates from the growing organs. It consists of the sink strength of the vegetative plant part and that of the fruits. The sink strength of a fruit depends on the age of the fruit and the cultivar. The sink strength of a fruit is highest around three weeks after anthesis. Sink strength is to some extent influenced by the number of seeds. A higher seed number increases sink strength, but the maximum is already reached at 50 seeds. The number of seeds increases with higher relative humidity and shows an optimum response to temperature. The vegetative sink strength increases with increasing temperature. A high plant sink strength causes the abortion of flowers and fruits. The cycle of high fruit set →

high sink strength → low fruit set → low sink strength → high fruit set explains the fluctuations in fruit set and consequently fruit yield. Another factor causing abortion is the export of auxin from the seeds. Auxin export from a fruit prevents the build-up of an abscission layer in the fruits and blocks auxin export from younger fruits. Prior to abortion, auxin exports from the reproductive organ diminishes, ethylene production increases, and lower activity of sucrose cleaving enzymes is found.

The sink strength of a fruit can be quantified by means of the potential fruit growth rate. Chapter 3 shows how these fruit growth curves are derived. A cultivar and a temperature experiment were conducted, in which fruits were grown with little or no competition (1-3 fruits per plant). Twice a week between anthesis and harvest, the length and circumference or diameter of these fruits were measured non-destructively. The estimated fruit volume was calculated based on dimensions of the fruit. Fruit volume was converted into fresh weight and subsequently into dry weight using two intermediate functions. A linear relationship was fitted between fruit fresh weight and estimated fruit volume. A Ricker, bi-exponential or polynomial function was established relating dry matter fraction of the fruit to fruit age. Logistic, Gompertz, Richards and beta growth functions were compared for time patterns of the non-destructively determined fruit dry weight. However, dry weight had not yet reached a plateau at the moment of harvesting and therefore fitting a sigmoid function resulted in high uncertainty in the estimated asymptote. Initial fruit growth was also fitted poorly. The sigmoid function was therefore fitted through the fresh weight. Dry weight over time was obtained by multiplying the sigmoid function for fresh weight by the function relating fraction fruit dry matter to fruit age. The Richards function gave the best fit for each data set, closely followed by the Gompertz function. Temperature did not influence final potential fruit size, while cultivar highly influenced potential fruit growth.

Chapter 4 examines the fruit set and yield patterns of six cultivars. Fruit fresh weight of the different cultivars ranged between 20 and 205 g per fruit. The variation in weekly fruit set, defined as the coefficient of variation was higher for cultivars with larger-sized fruits. The variation in weekly fruit yield was highly correlated with the variation in weekly fruit set. Fruit yield patterns were correlated with the lagged fruit set patterns, where the lag time was approximately the average fruit growth duration. Total plant growth, an approximation for source strength, was the same for all cultivars. It was assumed that the differences in fruit set patterns were caused by differences in the sink strength of a fruit.

This assumption was evaluated in Chapter 5. The source strength was obtained via model calculations with the measured leaf area, radiation, CO₂ and temperature. There were no large differences in source strength between the cultivars. Fruit sink strength was obtained from measurements as described in Chapter 3. The vegetative sink strength was estimated by iteration from the dry matter partitioning into the fruits, the simulated source strength and the calculated generative sink strength. Source-sink ratio was calculated from the source strength and the sum of the vegetative and generative sink strengths. The higher the potential fruit size of the cultivar, the lower the percentage fruit set and the higher the weekly variation in fruit set. Furthermore, the average ratio between actual and potential fruit size was higher for the cultivars with large-sized fruits. The average source-sink ratio at fruit set was also higher for the cultivars with the larger fruits. Using a simple simulation model, the effect of the differences in the various parameters was investigated. It was

concluded that not only a difference in the sink strength of the fruits, but also the differences in the threshold source-sink ratio required for fruit set were needed to explain the differences in fruit set pattern between the cultivars.

In Chapter 6, the abortion rate and the factors influencing this rate were quantified using survival analysis. Dates of anthesis, abscission and harvest were collected in a planting density experiment (2.5, 3.8 and 5.0 plants m⁻²) and the temperature experiment from Chapter 3. The survival times analysed were the times between anthesis and abscission, harvest or the end of the experiment. The first one is a realized survival time, whereas the latter two were censored. A lag time of 50°Cd was assumed between abortion and abscission. The start of every flower was shifted to 50°Cd before anthesis. A random effect (frailty) accounted for plant-to-plant differences in abortion. Initially, source and sink strength were used as linear time-dependent covariates. This analysis was improved by fitting a polynomial relation. In the density experiment, a higher source strength, up to 6 g CH₂O d⁻¹, decreased the abortion rate, but a source strength above this level did not decrease the abortion rate any further. A higher sink strength of the plant increased the abortion rate, but its effect was diminishing at values higher than 10 g CH₂O d⁻¹. However, correlations existed between source strength and sink strength. This gave some unexpected results at very low or very high values of source strength. The baseline abortion rate indicated that most flowers aborted around 100°Cd after anthesis. Adding the position of the fruit on the plant (main or side branch) improved the analysis. As an alternative for sink strength, the number of fruits divided into age classes of 50°Cd wide was used. Estimating the effect of each age class separately indicated that the relative impact of the age classes was similar to the effect of age on sink strength. Time-dependent covariates derived from source strength and sink strength, such as source-sink ratio, cumulative source-sink ratio, individual fruit weight, ratio between actual and potential fruit weight, were used as well, but did not yield a better model fit than the model with source strength and sink strength.

The results of the survival analysis of Chapter 6 were used in the simulations of Chapter 7. The baseline hazard was quantified by fitting a Gompertz function through the cumulative baseline hazard and taking the first derivative with respect to time. In the simulation the probability of abortion per day, as influenced by source strength and sink strength, was calculated for every fruit during the critical period of abortion (from 50°Cd before anthesis to 150°Cd after anthesis). The abortion rate was compared to a randomly drawn number from a uniform distribution between 0 and 1, and the flower or fruit aborted when the random number was lower than the calculated abortion rate. After calibration of the fruit abortion function, the simulation model simulated the fruit set patterns observed in the experiment on which the survival analysis was based. Adding the effect of the position of the flower (main or side shoot) did not improve the simulations of fruit set. A validation experiment with different planting densities gave good results as well, although in both density experiments a period with low fruit set in the beginning of the cultivation did not appear in the simulation results. The fruit set pattern of an experiment in a semi-closed greenhouse was simulated reasonably, but one peak in fruit set was missed. Validation with data from a commercial grower showed less satisfying results: overestimation in the beginning, followed by the correct pattern but with too high fruit set. In general, the simulated variation in fruit set of the density experiments in the total number of fruits set as well as in the weekly variation of fruit set was lower than the variation observed. This can be due to other sources of variation between plants, e.g. differences in leaf area or leaf

photosynthesis. A case study with constant source strength did not result in fluctuations in fruit set when averaged over 100 simulations, although individual plants showed fluctuations. Hence, the variation in source strength is the driving force for synchronization of fruit set between the plants.

The general discussion, Chapter 8, critically reflects on the results. It is concluded that survival analysis served as a proper tool for the derivation of the abortion function. Variation was an intrinsic part of the model, variation in input or weather data was not needed to obtain the variation. In general, the model was able to simulate the fruit set pattern observed. The variation in model output offers the opportunity to conduct ‘virtual experiments’ in order to assess the variability of the output variables under specific circumstances.

Samenvatting

Gewasgroeimodellen worden veel gebruikt in het onderzoek en het onderwijs. Ook op tuinbouwbedrijven neemt het gebruik van gewasgroeimodellen toe. De meeste gewasgroeimodellen zijn deterministisch. Een deterministisch model geeft bij bepaalde parameterwaarden en omgevingscondities als invoer altijd dezelfde uitkomst. In werkelijkheid is er echter sprake van variatie tussen de planten binnen een gewas. Het zou dus realistischer zijn om ook in modellen de gewasgroei met variatie te simuleren. Het doel van dit proefschrift is het inbrengen van een stochastisch onderdeel in een dynamisch gewasgroeimodel. Op deze manier verschilt de uitkomst per simulatie en is het mogelijk om de gemiddelde uitkomst te berekenen en de spreiding rond dit gemiddelde te bepalen.

In Nederland wordt het gewas paprika (*Capsicum annuum*) bijna jaarrond in kassen geteeld. Gedurende de teelt worden grote fluctuaties in de oogst waargenomen. De fluctuaties in oogst worden veroorzaakt door afwisselende perioden met hoge en lage vruchtabortie. Concurrentie om assimilaten tussen bloemen en vruchten is een belangrijke oorzaak van abortie: de aanwezigheid van snelgroeïende vruchten zorgt voor abortie van bloemen en jonge vruchten. Naast deze fluctuaties van abortie in de tijd, is er ook variatie in abortiepatronen: het aantal gezette vruchten en de positie van deze vruchten aan de plant verschilt tussen de planten. Door deze variatie in vruchtzetting tussen de planten is vruchtzetting in paprika's een goede casus voor het inbrengen van een stochastische component in een gewasgroeimodel. De statistische methode die de tijd tot een bepaalde gebeurtenis analyseert, survival analyse, is gebruikt om stochasticiteit in het simulatiemodel in te brengen. Deze methode analyseert de tijden tot een bepaalde gebeurtenis en relateert deze aan factoren die de gebeurtenis beïnvloeden. De hypothese is dat deze methode leidt tot een goede simulatie van de waargenomen vruchtzettingspatronen en de daarin waargenomen variatie.

In Hoofdstuk 2 is een inventarisatie gemaakt van de in de literatuur aanwezige kennis over de fasen waarin de bloemen en vruchten gevoelig zijn voor abortie en welke factoren abortie beïnvloeden. Hele jonge bloemknoppen (<2,5 mm), bloemknoppen die bijna open gaan, bloemen en vruchten tot 14 dagen na bloei zijn gevoelig voor abortie. 'Source sterkte' en 'sink sterkte' zijn belangrijke factoren die abortie beïnvloeden. Source sterkte is het aanbod van assimilaten, afkomstig van de fotosynthese, dat gebruikt kan worden voor groei. Bij een hogere source sterkte aborteren er minder vruchten. Een hogere source sterkte kan gerealiseerd worden door het stralingsniveau en/of het CO₂-niveau te verhogen of door de plantdichtheid te verlagen. Tekort aan water of nutriënten verlaagt de source sterkte. Een hogere temperatuur verhoogt in eerste instantie de source sterkte, maar boven de 30 °C daalt de source sterkte weer. 'Sink sterkte' is de vraag naar assimilaten van de groeiende organen. De sink sterkte is onder te verdelen in de sink sterkte van de vegetatieve organen en de sink sterkte van de vruchten. De sink sterkte van een vrucht hangt af van zijn leeftijd en van het ras. De sink sterkte is het grootst drie weken na de bloei. De sink sterkte wordt tot op zekere hoogte bepaald door het aantal zaden. Meer zaden vergroten de sink sterkte, maar het maximum is al bereikt bij circa 50 zaden. Een hogere

relatieve luchtvochtigheid zorgt voor hogere zaadzetting. Zaadzetting laat een optimum zien bij een stijgende temperatuur. De sink sterkte van de vegetatieve delen neemt toe met een toenemende temperatuur. Als de sink sterkte van een plant toeneemt, aborteren er meer bloemen en jonge vruchten. De cyclus van hoge vruchtzetting → hoge sink sterkte → lage vruchtzetting → lage sink sterkte → hoge vruchtzetting verklaart de fluctuaties in vruchtzetting en daardoor ook de fluctuaties in oogst. Daarnaast spelen ook hormonen, met name auxine, een rol bij de abortie. Auxine wordt geproduceerd door de zaden. De export van auxine uit de vrucht voorkomt de opbouw van een abscissielaag in de vruchtsteel. Tegelijkertijd blokkeert de export van auxine uit een reeds bestaande vrucht de export van auxine uit nieuwe vruchten. Voordat een vrucht aborteert, wordt er in die vrucht een verlaging van de export van auxine waargenomen, een verhoogde productie van ethyleen en een lagere activiteit van sucrose-verwerkende enzymen.

De sink sterkte van een vrucht kan worden gekwantificeerd met de potentiële vruchtgroeisnelheid. Hoofdstuk 3 laat zien hoe deze vruchtgroeisnelheid wordt bepaald. Er is een rassenexperiment met 6 rassen en een experiment met 3 temperaturen (gemiddelde dagelijkse temperatuur 18, 21 and 24 °C) uitgevoerd. In deze experimenten groeiden de vruchten met geen tot weinig concurrentie van andere vruchten (1 tot 3 vruchten per plant). Twee keer per week tussen de bloei en de oogst van de vrucht werden de lengte en de omtrek of de diameter van de vrucht gemeten. Uit deze metingen werd het geschatte vruchtvolume berekend. Uit dit vruchtvolume werd achtereenvolgens het versgewicht van de vrucht en het drooggewicht van de vrucht berekend met behulp van twee functies. De eerste functie was een lineaire functie tussen het versgewicht van de vrucht en het geschatte vruchtvolume. De tweede functie was een polynoom, een Ricker functie, of een bi-exponentiële functie die het drogestof gehalte van de vrucht relateerde aan de leeftijd van de vrucht. Vier sigmoïde functies, de Logistische, Gompertz, Richards en beta-groei functie, werden door het geschatte drooggewicht over de tijd gefit en vergeleken. Het bleek echter dat het drooggewicht nog niet gestabiliseerd was op het moment van oogst. Dit zorgde voor een grote onzekerheid in de waarde van de gefitte asymptoot. Ook het begin van de curve werd slecht gefit. De sigmoïde functies werden daarom door het versgewicht gefit, wat wel stabiliseerde tegen het einde van de groeiperiode. De Richards functie gaf de beste fit, op de voet gevolgd door de Gompertz functie. Temperatuur had geen invloed op het uiteindelijke vruchtgewicht, maar ras had een duidelijke invloed. Een functie voor het drooggewicht van de vrucht over de tijd kan worden verkregen door de sigmoïde functie te vermenigvuldigen met de functie die het drogestof gehalte van de vrucht relateert aan de vruchtleeftijd.

In Hoofdstuk 4 zijn de vruchtzettings- en oogstpatronen van zes verschillende *Capsicum* rassen bekeken. Het versgewicht van de vruchten van de zes rassen varieerde tussen 20 en 205 gram. De variatie in wekelijkse vruchtzetting, gedefinieerd als de coëfficiënt van variatie, was hoger voor rassen met grotere vruchten. De variatie in wekelijkse vruchtoogst was hoog gecorreleerd met de variatie in wekelijkse vruchtzetting. De patronen in vruchtoogst waren gecorreleerd met de verschoven vruchtzettingspatronen, waarbij de verschuiving rond de gemiddelde vruchtgroeiduur lag. De totale biomassagroei van de planten, een maat voor de source sterkte, was gelijk voor alle rassen. Dit leidde tot de vooronderstelling dat de verschillen in vruchtzettingspatronen tussen de rassen veroorzaakt werden door verschillen in de sink sterkte van de vrucht.

Deze vooronderstelling is onderzocht in Hoofdstuk 5. De dagelijkse source sterkte werd bepaald met modelsimulaties waarbij het gemeten bladoppervlakte, de dagelijkse straling, het CO₂-niveau en de temperatuur inputgegevens van het model waren. Er waren geen grote verschillen in source sterkte tussen de rassen. De sink sterkte van de vruchten is bepaald aan de hand van de metingen in Hoofdstuk 3. De vegetatieve sink sterkte werd door middel van iteratie bepaald uit de in de vruchten aanwezige drogestof als fractie van de totale drogestofproductie, de gesimuleerde source sterkte en de berekende sink sterkte van de vruchten. De dagelijkse source-sink ratio is berekend als de source sterkte gedeeld door het totaal van de vegetatieve en generatieve sink sterkte. Hoe groter de potentiële vruchtgrootte van het ras was, des te lager het percentage vruchtzetting was. Een grotere potentiële vruchtgrootte resulteerde ook in grotere fluctuaties in de wekelijkse vruchtzetting. De ratio tussen het gerealiseerde en potentiële vruchtgewicht was hoger voor de rassen met hogere potentiële vruchtgrootte, evenals als de benodigde source-sink ratio voor vruchtzetting. Met een eenvoudig simulatiemodel is het effect van de verschillen in parameters tussen de rassen bekeken. Hieruit bleek dat niet alleen de verschillen in sink sterkte van de vrucht nodig waren om de verschillen in vruchtzettingsspatronen tussen rassen te verklaren, maar dat ook de minimum source-sink ratio die nodig is voor vruchtzetting hiervoor van belang is.

In Hoofdstuk 6 zijn de abortiesnelheid en de factoren die deze snelheid beïnvloeden gekwantificeerd. In een plantdichtheidsexperiment met drie plantdichtheden (2,5, 3,8 en 5,0 planten per m²) en het temperatuursexperiment uit Hoofdstuk 3 werden waarnemingen gedaan aan bloeidatum, datum van abscissie en oogstdatum. De tijden tussen bloei en abscissie, oogst of einde van het experiment zijn de geanalyseerde abortietijden. De eerste is een echte overlevingstijd, de andere twee zijn zogenaamde censored overlevingstijden. Om te corrigeren voor de tijd tussen abortie en abscissie werd een tijdsspanne van 50 °Cd tussen deze twee gebeurtenissen verondersteld. Het startpunt van elke bloem werd verschoven naar 50 °Cd voor de bloei. In de analyse werd een aselechte factor (frailty) meegenomen om verschillen in abortiesnelheid tussen de planten te onderscheiden. Source sterkte en sink sterkte werden eerst als lineaire, tijdsafhankelijke factoren in de survival analyse meegenomen. De analyse verbeterde als deze factoren niet lineair maar als polynomen werden gefit. In het plantdichtheidsexperiment zorgde een toename van de source sterkte tot 6 g CH₂O per dag per plant ervoor dat de abortiesnelheid afnam; hogere waarden zorgden niet voor een verdere afname in de abortiesnelheid. Een hogere sink sterkte vergrootte de abortiesnelheid, maar het effect nam af boven de 10 g CH₂O per dag per plant. Er waren correlaties tussen source en sink sterkte, wat ervoor zorgde dat bij hele hoge of lage waarden van de source sterkte en sink sterkte er onverwachte effecten optraden. De basisabortiesnelheid, dat wil zeggen de abortiesnelheid in afwezigheid van source en sink sterkte, gaf aan dat de meeste bloemen en vruchten rond 100 °Cd na bloei aborteerden. De analyse verbeterde als de positie van de bloem of vrucht, die aangeeft of de bloem op de hoofdtak of op de zijtak zat, werd toegevoegd aan de analyse. Als alternatief voor de sink sterkte werden de leeftijden van de aanwezige vruchten ingedeeld in klassen met een breedte van 50 °Cd. Analyse met deze leeftijdsklassen liet zien dat het relatieve effect van de leeftijdsklassen de sink sterkte goed benaderde. Andere tijdsafhankelijke factoren die van source en sink sterkte zijn afgeleid, zoals source-sink ratio, cumulatieve source-sink ratio, gewicht van de vrucht en de ratio tussen gerealiseerd en potentieel

vruchtgewicht, zijn ook gebruikt in de analyse, maar deze gaven geen betere fit dan wanneer source en sink sterkte als afzonderlijke factoren werden meegenomen.

In Hoofdstuk 7 werden de resultaten van de survival analyse in Hoofdstuk 6 gebruikt om abortie van paprika bloemen en vruchten te simuleren. De cumulatieve basissnelheid van abortie werd gekwantificeerd door er een Gompertz functie doorheen te fitten. Door de afgeleide hiervan te nemen is de basissnelheid van abortie verkregen. In de simulaties wordt de abortiesnelheid per dag berekend afhankelijk van de source en sink sterkte. Dit gebeurt per bloem tijdens de kritieke periode voor abortie, die loopt van 50 °Cd voor bloei tot 150 °Cd na bloei. Deze abortiesnelheid werd vergeleken met een random getal uit een uniforme verdeling tussen 0 en 1. De bloem of vrucht aborteerde wanneer dit random getal kleiner was dan de abortiesnelheid. Na calibratie simuleerde het model de waargenomen patronen in vruchtzetting van het dichtheidsexperiment waarop de survival analyse was gebaseerd goed. Het toevoegen van de positie van de bloem (hoofd- of zijtak) gaf geen verbetering van de simulatie. Een validatie-experiment met verschillende plantdichtheden werd ook goed gesimuleerd, al hadden beide dichtheidsexperimenten een periode van lage vruchtzetting die niet goed gesimuleerd werd. Het vruchtzettingspatroon van een experiment in een half-gesloten kas werd redelijk gesimuleerd, maar een piek in vruchtzetting miste. Validatie met gegevens van een commerciële teler gaf minder bevredigende resultaten. Er was overschatting in het begin, waarna het patroon juist werd gesimuleerd, maar met te hoge waarden. De gesimuleerde variatie, zowel in het totale aantal vruchten per plant als de wekelijkse variatie in vruchtzetting, was lager in de simulaties dan in werkelijkheid. Dit kan komen omdat er nog andere bronnen van variatie zijn tussen de planten, zoals verschillen in bladoppervlakte of fotosynthesesnelheid. Een studie waarin gesimuleerd is met constante source sterkte liet zien dat er gemiddeld over 100 simulaties geen fluctuaties waren, maar dat elke plant afzonderlijk wel fluctuaties in vruchtzetting had. Hieruit is geconcludeerd dat de variatie in source sterkte een belangrijke drijvende kracht is achter de synchronisatie van de vruchtzetting tussen de planten.

In de algemene discussie, Hoofdstuk 8, wordt kritisch gereflecteerd op de behaalde resultaten. Er wordt geconcludeerd dat survival analyse een juiste methode is om de abortie functie te bepalen. Variatie is nu een intrinsiek onderdeel van het simulatiemodel, wat ontstaat tijdens de simulaties zonder dat er andere inputwaarden of weersgegevens nodig zijn. Het model kon in het algemeen de waargenomen patronen bevredigend simuleren. Variatie in de simulatie-uitkomsten biedt de mogelijkheid om ‘virtuele experimenten’ uit te voeren, waarin de variatie van de modeluitkomsten onder bepaalde omstandigheden kan worden bestudeerd.

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Curriculum Vitae

Ageeth Maaïke Wubs was born on October 28th, 1979 in Schiedam, the Netherlands. She attended 'Stedelijk Gymnasium Schiedam' for her secondary education, from which she graduated in 1998. In the same year, she commenced her study 'Plantenteeltwetenschappen' (Crop Sciences) at Wageningen University. She discovered that it was possible to combine her interest in plants with more quantitative methods, and specialised in systems analysis, crop growth models and population dynamics. For her first thesis, she stayed four months at the field station of the International Centre for Insect Physiology and Ecology in Port Sudan, Sudan. She studied the effect of nitrogen content of the food on the growth and development of desert locust (*Schistocerca gregaria*). During the academic year 2002-2003, she set aside her studies to participate one year fulltime in the Student Council. In this Council, she was active in different committees, dealing among others with the university policy on education. The following year, she resumed her studies and conducted a thesis on the relative leaf area model for crop-weed competition. She finished her MSc-study with an internship at Rothamsted Research, Harpenden, UK, where she conducted experiments, analysis and simulations for the conductance model on crop-weed competition. She graduated in 2004. During the following year she worked on a short project on modelling intercropping in Africa. In November 2005, she started the PhD project which is described in this thesis. From January 2010 onwards, she participates as a post-doc researcher in the EU-project SPICY, working on integration of QTL's in simulation models.

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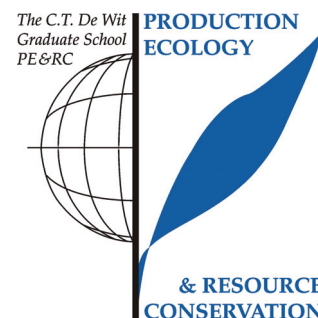
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- Abortion of reproductive organs in sweet pepper (*Capsicum annuum* L.): a review (2006)

Post-Graduate Courses (11.5 ECTS)

- Uncertainty modelling and analysis; WIMEK/SENSE (2006)
- Complex population dynamics; Crop and Weed Ecology (2006)
- Advanced statistics; PE&RC (2008)

Deficiency, Refresh, Brush-up Courses (2.8 ECTS)

- Basic statistics; PE&RC (2006)
- Greenhouse technology; Farm Technology (2006)

Competence Strengthening / Skills Courses (3.3 ECTS)

- PhD Competence assessment; WGS (2006)
- Writing and presenting a scientific publication; WGS (2009)
- Teaching methodology and skills for PhD Students; Docenten Ondersteuning, WUR (2009)

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- PE&RC Day "The truth of science" (2005)
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- Annual meeting of the NVTB, Schoorl (2007)
- 40 years Theory and models at Wageningen UR (2008)

International Symposia, Workshops and Conferences (5 ECTS)

- Workshop functional structural modelling; Wageningen, the Netherlands (2006)
- Hortimodel 2006; Wageningen, the Netherlands (2006)
- Greensys 2009; Quebec, Canada (2009)

Courses in Which the PhD Candidate Has Worked as a Teacher

- Practicum Crop Ecology; Tuinbouwketens, 10 days (2006 / 2007/ 2008)
- Practicum Inleiding Statistiek; Wiskundige en Statistische Methoden, 24 days (2007 / 2008)

Supervision of MSc Student (1 student; 8 days)

- Fruit set and yield patterns of sweet pepper under different temperatures

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