Effect of temperature and photoperiod on broccoli development, yield and quality in south-east Queensland

By

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DECLARATION OF ORIGINALITY

This thesis reports the original work of the author, except otherwise acknowledged.

It has not been submitted previously at this or any other University.

Daniel K.Y. Tan
ABSTRACT

Broccoli is a vegetable crop of increasing importance in Australia, particularly in south-east Queensland and farmers need to maintain a regular supply of good quality broccoli to meet the expanding market. However, harvest maturity date, head yield and quality are all affected by climatic variations during the production cycle, particularly low temperature episodes. There are also interactions between genotype and climatic variability. A predictive model of ontogeny, incorporating climatic data including frost risk, would enable farmers to predict harvest maturity date and select appropriate cultivar – sowing date combinations.

The first stage of this research was to define floral initiation, which is fundamental to predicting ontogeny. Scanning electron micrographs of the apical meristem were made for the transition from the vegetative to advanced reproductive stage. During the early vegetative stage (stage 1), the apical meristem was a small, pointed shoot tip surrounded by leaf primordia. The transitional stage (stage 2) was marked by a widening and flattening to form a dome-shaped apical meristem. In the floral initiation stage (stage 3), the first-order floral primordia were observed in the axils of the developing bracts. Under field conditions, the shoot apex has an average diameter of $500 \pm 3 \, \mu m$ at floral initiation and floral primordia can be observed under a light microscope.

Sub-zero temperatures can result in freezing injury and thereby reduce head yield and quality. In order to predict the effects of frosts, it is desirable to know the stages of development at which plants are most susceptible. Therefore, the effects of sub-zero temperatures on leaf and shoot mortality, head yield and quality were determined after exposure of plants to a range of temperatures for short periods, at different stages of development (vegetative, floral initiation and buttoning). Plants in pots and in the field were subjected to sub-zero temperature regimes from $-1 \, ^\circ C$ to $-19 \, ^\circ C$. Extracellular ice formation was achieved by reducing temperatures slowly, at a rate of $-2 \, ^\circ C$ per hour. The floral initiation stage was most sensitive to freezing injury, as yields were significantly reduced at $-1 \, ^\circ C$ and $-3 \, ^\circ C$, and shoot apices were killed at $-5 \, ^\circ C$. There was no significant yield reduction when the inflorescence buttoning
stage was subjected to –1 °C and –3 °C. Although shoot apices at buttoning survived the –5 °C treatment, very poor quality heads of uneven bud size were produced as a result of arrested development. The lethal temperature for pot-grown broccoli was between –3 °C and –5 °C, whereas the lethal temperature for field-grown broccoli was between –7 °C and –9 °C. The difference was presumably due to variation in cold acclimation. Freezing injury can reduce broccoli head yield and quality, and retard plant growth. Crop development models based only on simple thermal time without restrictions will not predict yield or maturity if broccoli crops are frost-damaged.

Field studies were conducted to develop procedures for predicting ontogeny, yield and quality. Three cultivars, (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) were sown on eight dates from 11 March to 22 May 1997, and grown under natural and extended (16 h) photoperiods in a sub-tropical environment at Gatton College, south-east Queensland, under non-limiting conditions of water and nutrient supply. Daily climatic data, and dates of emergence, floral initiation, harvest maturity, together with yield and quality were obtained. Yield and quality responses to temperature and photoperiod were quantified. As growing season mean minimum temperatures decreased, fresh weight of tops decreased while fresh weight harvest index increased linearly. There was no definite relationship between fresh weight of tops or fresh weight harvest index and growing season minimum temperatures ≥ 10 °C. Genotype, rather than the environment, mainly determined head quality attributes. ‘Fiesta’ had the best head quality, with higher head shape and branching angle ratings than ‘Greenbelt’ or ‘Marathon’. Bud colour and cluster separation of ‘Marathon’ were only acceptable for export when growing season mean minimum temperatures were < 8 °C. Photoperiod did not influence yield or quality in any of the three cultivars. A better understanding of genotype and environmental interactions will help farmers optimise yield and quality, by matching cultivars with time of sowing.

Crop developmental responses to temperature and photoperiod were quantified from emergence to harvest maturity (Model 1), from emergence to floral initiation (Model 2), from floral initiation to harvest maturity (Model 3), and in a combination of Models 2 and 3 (Model 4). These thermal time models were based on optimised base
and optimum temperatures of 0 and 20 °C, respectively. These optimised temperatures were determined using an iterative optimisation routine (simplex). Cardinal temperatures were consistent across cultivars but thermal time of phenological intervals were cultivar specific. Sensitivity to photoperiod and solar radiation was low in the three cultivars used. Thermal time models tested on independent data for five cultivars (‘Fiesta’, ‘Greenbelt’, ‘Marathon’, ‘CMS Liberty’ and ‘Triathlon’) grown as commercial crops on the Darling Downs over two years, adequately predicted floral initiation and harvest maturity.

Model 4 provided the best prediction for the chronological duration from emergence to harvest maturity. Model 1 was useful when floral initiation data were not available, and it predicted harvest maturity almost as well as Model 4 since the same base and optimum temperatures of 0 °C and 20 °C, respectively, were used for both phenological intervals. Model 1 was also generated using data from 1979-80 sowings of three cultivars (‘Premium Crop’, ‘Selection 160’ and ‘Selection 165A’). When Model 1 was tested with independent data from 1983-84, it predicted harvest maturity well. Where floral initiation data were available, predictions of harvest maturity were most precise using Model 3, since the variation, which occurred from emergence to floral initiation, was removed. Prediction of floral initiation using Model 2 can be useful for timing cultural practices, and for avoiding frost and high temperature periods.

This research has produced models to assist broccoli farmers in crop scheduling and cultivar selection in south-east Queensland. Using the models as a guide, farmers can optimise yield and quality, by matching cultivars with sowing date. By accurately predicting floral initiation, the risk of frost damage during floral initiation can be reduced by adjusting sowing dates or crop management options. The simple and robust thermal time models will improve production and marketing arrangements, which have to be made in advance. The thermal time models in this study, incorporating frost risk using conditional statements, provide a foundation for a decision support system to manage the sequence of sowings on commercial broccoli farms.
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6.1 Head yield and quality attributes of five broccoli grades (Export Japan, Export South-east Asia, Domestic Chain Stores, Domestic Central Markets Large and Small), expressed as head fresh weight (g head⁻¹), head diameter (mm), head shape (1-5), branching angle (1-5) and cluster separation (1-5) ratings packed by eight packers in a packing house near Brookstead, south-east Queensland. Means of grades are averaged over eight packers (n = 40). L.s.d. values are at P=0.05 using Fisher’s protected l.s.d. tests for grade main effect. Means followed by the same letter within the same row are not significantly different at P=0.05.

6.2 Head quality attributes of three broccoli cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’), expressed as head shape (1-5), branching angle (1-5), cluster separation (1-5) and bud evenness (1-5) ratings, bud size (mm), bractiness (number of bracts protruding through head), percent head dry weight (%) (dry/fresh weight), and principal components 1 and 2 (PC₁ & PC₂) grown under a range of photoperiod and temperature regimes at Gatton College, south-east Queensland. Means of cultivars are averaged over two photoperiods (natural and 16 h) and eight sowing dates (n = 48). L.s.d values are at P=0.05, using Fisher’s protected l.s.d. tests for the cultivar main effect. Means followed by the same letter within the same row are not significantly different at P=0.05.

7.1 Main and interactive effects of photoperiod extension (PP), sowing date (SD) and cultivar (CV) on the chronological time (days), thermal time (°C d) and accumulated solar radiation (MJ m⁻²) during the interval from emergence to floral initiation, and total leaf number in broccoli [**, *, n.s. for P<0.01, P<0.05, not significantly different (P=0.05) respectively]. Dash (-) indicates no l.s.d. was calculated as the F-test was not significant at P=0.05.
7.2 Main and interactive effects of photoperiod extension (PP), sowing date (SD) and cultivar (CV) on the chronological time (days), thermal time (°C d), accumulated solar radiation (MJ m$^{-2}$), and effective thermal time (ETT) during the interval from floral initiation to harvest maturity (FIHM) in broccoli [**, *, n.s. for P<0.01, P<0.05, not significantly different (P=0.05) respectively]. Dash (-) indicates no l.s.d. was calculated as the F-test was not significant at P=0.05.

7.3 Optimum rate of development [expressed as rate of progress (day$^{-1}$), and chronological time (days)] and thermal time (TT °C d, mean ± s.e.) duration with optimised base and optimum temperatures of 0 and 20 °C during the time from emergence to floral initiation for three broccoli cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) grown under a range of photoperiod and temperature regimes in a field experiment at Gatton College, south-east Queensland.

7.4 Duration (mean ± s.e.) from floral initiation to harvest maturity, expressed as chronological time (days), thermal time (°C d), accumulated solar radiation (MJ m$^{-2}$), and effective thermal time (ETT) for three broccoli cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) grown under a range of photoperiod and temperature regimes in a field experiment at Gatton College, south-east Queensland, as estimated from optimisation routines using DEVEL. Thermal time was calculated using base and optimum temperatures of 0 and 20 °C. ETT was calculated using $a$ values of 0.045, 0.039 and 0.354 for ‘Fiesta’, ‘Greenbelt’ and ‘Marathon’. Means given are of 48 experimental units.

8.1 Main and interactive effects of photoperiod extension (PP), sowing date (SD) and cultivar (CV) on the chronological time (days) and thermal time (°C d) duration of the interval from emergence to harvest maturity in broccoli [**, *, n.s. for P<0.01, P<0.05, not significantly different (P=0.05) respectively]. Dash (-) indicates no l.s.d. was calculated as the F-test was not significant at P=0.05.

8.2 Duration (mean ± s.e.) from emergence to floral initiation (EFI), from floral initiation to harvest maturity (FIHM) and from emergence to harvest maturity (EHM) for three broccoli cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) expressed as thermal time (°C d) grown under a range of photoperiod and temperature regimes in a field experiment at Gatton College, south-east Queensland, as estimated from optimisation routines using DEVEL. Thermal time was calculated from base and optimum temperatures of 0 and 20 °C. Means given were of 48 experimental units.
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<td>Dry weight harvest index</td>
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<td>h</td>
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<td>ha</td>
<td>Hectare</td>
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<tr>
<td>HFW</td>
<td>Head fresh weight (g)</td>
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<td>HM</td>
<td>Harvest maturity</td>
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<td>l.s.d.</td>
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<td>LT$_{50}$</td>
<td>Killing temperature for 50% of the population (°C)</td>
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<td>r$^2$</td>
<td>Coefficient of determination</td>
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<td>REC</td>
<td>Relative electrical conductivity</td>
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<td>RMSD</td>
<td>Root mean square deviation</td>
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<td>TTC</td>
<td>Triphenyl tetrazolium chloride</td>
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Terminology

Throughout this thesis, the following definitions of terms (Birch 1996) will be used.

**Coefficient**  
Derived constant that appears in an equation. It may be a parameter.

**Plant development**  
The change within an interval from one phenological event to another, and from one phenological interval to another in a plant.

**Fitted value**  
The value of a dependent variable determined by substitution in a regression derived from data collected in one of the experiments reported in this thesis. Other parts of the word ‘fit’ are to be taken to have similar application.

**Ontogeny**  
The sequence of events that constitute the life cycle of the plant from sowing until harvest maturity is reached (Birch 1996).

**Phenology**  
Study of periodic biotic events that occur once in a growing season of a crop. It describes and measures developmental process, physiological processes controlling growth and development, and the environment (Alm et al. 1991).

**Parameter**  
A constant for a simulation that characterises an element of a system. It is constant for a specific location or application or time period.

**Prediction**  
The output value of a state variable provided by a model, other parts of the word ‘predict’ are to be taken to have similar application.
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I dedicate this thesis to my beautiful, loving wife, Xiu Zhen (Lily) and appreciate her continuing support and sacrifices made. I also acknowledge the support and encouragement of my parents and sister, Christina, during this research.
Chapter 1

General Introduction

1.1 Broccoli in Australia

Broccoli (*Brassica oleracea* L. var. *italica* Plenck) has become an important crop in Australia since the introduction of hybrid cultivars in the early 1970s. Hybrid cultivars introduced a degree of uniformity to the marketed broccoli inflorescence (hereafter called head), which has enabled the development of the current fresh and export markets. A five-fold increase in the area planted and a six-fold increase in production has occurred from 1982 to 1997 (Deuter 1995, Australian Bureau of Statistics 1998). The broccoli industry in 1997 produced 40,546 t (metric tons) of product Australia-wide from 6,961 ha, of which 9,116 t were produced in Queensland, from 1,619 ha (Australian Bureau of Statistics 1998). The major broccoli growing districts in south-east Queensland are the Lockyer Valley, Darling Downs and the Granite Belt. From July 1995 to June 1996, 8,183 t were exported from Australia with a gross value of A$17.6 million (Australian Bureau of Statistics 1996, pers. comm.). Recently, broccoli gained further popularity following the discovery that it contains glucoraphanin, a precursor to the cancer-fighting chemical, sulforaphane, and other anti-carcinogenic isothiocyanates (mustard oils) (Fahey *et al.* 1997, Wolton 1998).

1.2 Systems approach to define the problem

A well-planned production or crop scheduling program, is essential to maintain continuity of supply for domestic and export marketing from May to September for crops in south-east Queensland. The need to maintain a regular supply of good quality broccoli for export was a concern for a large commercial farmer on the Darling Downs, 200 km west of Brisbane (Tan *et al.* 1997). This farmer produces broccoli during this period by making over 30 separate sowings at 3-6 day intervals from early March to late May. Fertilisers are applied at rates determined from soil tests and rainfall is supplemented with furrow irrigation. When variable climatic conditions occurred, crops matured earlier or later than planned and produced poor
quality heads. The farmer approached the University of Queensland to investigate the problem.

When broccoli reaches optimum harvest time, it is sequentially hand harvested and transported in bulk bins to the packing house. In effect, harvesting is the first step in grading as immature heads or over-mature heads are not cut. At the packing house, broccoli is pre-cooled in a cold room, then further graded and packed into polystyrene cartons destined for specific markets. The cartons are stacked on pallets and transported by refrigerated trucks to domestic central markets or to ports for export. Broccoli exported to Japan and Taiwan is sea freighted whereas broccoli exported to Singapore and Malaysia is air freighted. Market agents for both domestic and export markets provide feedback to the farmer on the quality and timeliness of arrival of each consignment (Fig. 1.1). Information on timeliness and consistency of supply is especially valuable for export farmers who have to make advance bookings for air or sea transport and advise overseas customers of expected arrival dates. Difficulties arise when export orders are delayed or cancelled. Long term cold storage to even out supply is not possible because fresh broccoli is highly perishable (Klieber and Wills 1991).

Closer analysis of the production and distribution systems, in conjunction with the farmer, identified the crop production component (Fig. 1.1) as the major cause of irregular supply and poor quality. The other components were tightly controlled and potential problems were minimised by effective communication, personnel management and preventative maintenance. In an attempt to maintain regular harvests, farmers stagger sowing dates with cultivars of different maturity times (Pearson and Hadley 1988). Curvilinear relationships between chronological time (days) from sowing to maturity and sowing date for three cultivars (Fig. 1.2, see Chapter 8, Section 8.3.1) were developed in this study using the production schedules of sowing and harvest dates for seven years (1991, 1993 to 1998) maintained by the farmer. Unfortunately, such a production plan which is usually based on average climatic conditions lacks accuracy and is likely to fail in seasons that differ from the long-term average. For example, very cold winters in 1994 and 1995 delayed harvest and reduced broccoli quality on the Darling Downs, thus resulting in some farmers
being unable to supply their customers on schedule (Jauncey, P. 1996, pers. comm.). Conversely, periods of warmer than average weather hasten crop development leading to over-supply and low market prices.

Fig. 1.1. System for production and marketing of broccoli. The solid lines show the flow of broccoli through the system and the broken lines show the flow of information.

Production was the most crucial component based on the farmer’s experience and reports from the literature. Crop development is strongly affected by temperature (Wurr et al. 1991a, 1992, 1995). Good farm records for 1994 to 1998 (including sowing and harvest dates for each sowing) could be used for detailed analysis. Genotype and environment interactions could be studied to improve accuracy in predicting ontogeny and understanding of environmental effects on yield and quality.
Fig. 1.2. Effect of sowing date [Julian day (Jday) where 1 = 1 Jan] and cultivar, (a) ‘Fiesta’, (b) ‘Greenbelt’, and (c) ‘Marathon’ on chronological duration (days) from sowing to harvest maturity for broccoli grown on a commercial farm at Brookstead, south-east Queensland. The growing seasons (years) were 1996 (●), 1997 (■), and 1998 (*) for (a) and 1991 (●), 1993 (■), 1994 (*), 1995 (●), 1996 (◆) and 1997 (●) for (b) and (c).
1.3 Yield and quality

Broccoli yield and quality can be influenced by environmental conditions such as temperature during crop development (Titley 1985, 1987). There is limited information on the effect of temperature on yield and quality. Polynomial equations have been used to describe the relationships between growing season mean temperatures and quality attributes such as head shape, colour separation and bractiness (Dufault 1996). Researchers have speculated that yield and quality may be affected by photoperiod but no data were presented (Chung 1985a, Chung and Strickland 1986, Thompson and Taylor 1970). Yield and quality are important to growers, and cultivar and sowing date can have a major impact on marketable yield. Better understanding of genotype by environment interactions will help farmers optimise yield and quality, by matching cultivars to time of sowing.

Adverse temperature conditions such as severe frosts can reduce broccoli yield and quality (Tan et al. 1999). Yield and quality were downgraded after sub-zero temperatures were experienced in the winters of 1994 and 1995 in the eastern Darling Downs (Jauncey, P. 1996 pers. comm.). Severe frosts (-7 to -8 °C air temperature at 1.5 m) for 3 consecutive days in August 1995 retarded growth and resulted in poor head quality. Ideally, broccoli plants growing during winter should not only survive, but also resist freezing injury (Fuller et al. 1989). To predict effects of frost, it is necessary to know the stages of development at which plants are most susceptible to freezing injury.

1.4 Prediction of ontogeny and maturity

Broccoli farmers need to know in advance of a change in time of harvest maturity (see Chapter 2, Section 2.2.2.c) arising from variable climatic conditions so that their forward-marketing arrangements can be modified to reduce the economic effects of irregularity in supply. Thus, a computer model that predicts crop ontogeny could be a useful aid to management. A model of broccoli ontogeny can be developed with the measurement of ontogeny in different environments. Most research on crop scheduling involves time of sowing experiments with a range of cultivars. The choice
of cultivar is important because they vary in time to maturity and adaptability to prevailing temperatures. Thus, a cultivar needs to be matched with sowing time to ensure a regular supply of marketable heads (Titley 1981, 1985, 1987). Accurate prediction of crop ontogeny will also allow a farmer to supply irrigation, agrochemicals and fertiliser according to requirements of plants associated with phenological events rather than a fixed schedule. For example, if floral initiation is expected to occur earlier, side-dressings of fertiliser may be applied earlier while the crop is still in the vegetative phase (Tan et al. 1997).

Limited work has been done on crop scheduling and prediction of optimum harvest time for the popular broccoli cultivars such as ‘Greenbelt’ and ‘Marathon’ used in Australia. Existing models are either site specific (Chung 1981) or are complex, and developed for temperate cultivars grown in higher latitudes (Wurr et al. 1991a, 1992). Hence, there is a need to develop a robust model that is simple, precise and can be applied to a range of cultivars in different locations. Typically, a model of ontogeny has four parameters, based on (i) thermal time (Arnold 1959) calculated from (ii) base (T\text{base}), (iii) optimum (T\text{opt}) and (iv) maximum (T\text{max}) temperatures for each phenological interval, namely emergence to floral initiation and floral initiation to harvest maturity (Diputado and Nichols 1989, Fyffe and Titley 1989). An iterative optimisation technique was applied to data in the present study to derive T\text{base} and T\text{opt}, and a 2-stage broken linear temperature response (Fig 1.3a) best described the data (Holzworth and Hammer 1992). Since T\text{max} was not determined in the present study, the thermal time model used in this study has three parameters based on (i) thermal time calculated from (ii) T\text{base} and (iii) T\text{opt} of 0 and 20 °C, respectively (Chapter 7) using the equation:

\[
\text{Thermal time} = \left[ \left( T\text{Dmax} + T\text{Dmin} \right) / 2 \right] - T\text{base}
\]

where T\text{Dmax} = maximum temperature for the day, T\text{Dmin} = minimum temperature for the day. All T\text{Dmin} < T\text{base} were considered to be equal to 0 °C, and all T\text{Dmax} > T\text{opt} were considered to be equal to 20 °C (Barger System) (Arnold 1974, Titley 1985, 1987, Wurr et al. 1991a).

Many researchers working on the effect of temperature on broccoli have assumed that photoperiod does not have a modifying effect on floral initiation (Miller et al. 1985,
Miller 1988, Marshall and Thompson 1987a, 1987b). However, some Japanese researchers have reported that where broccoli was grown at 17 °C, long-day (16 h) conditions promoted floral initiation more effectively than short-day (8 h) conditions (Fujime et al. 1988). If the crop is sensitive to photoperiod, a broken linear photoperiod response (Fig. 1.3b) (Angus et al. 1981b, Birch 1996, Holzworth and Hammer 1992) can be incorporated into the general model.

1.5 Objectives of this research

This research was conducted to provide the foundation for a decision support system for broccoli production in south-east Queensland. Hence, the objectives of this study were to:

(i) provide a reliable and repeatable method of detecting floral initiation in broccoli;

(ii) describe and quantify the effects of sub-zero temperatures imposed at sequential developmental stages on leaf and shoot apex mortality, and head yield and quality;

(iii) quantify the response of head yield and quality to temperature and photoperiod in three cultivars grown under field conditions in a sub-tropical environment;

(iv) quantify the response of crop development to temperature and photoperiod in three cultivars from emergence to floral initiation, and from floral initiation to harvest maturity;

(v) compare and validate thermal time models from emergence to harvest maturity.
Fig. 1.3. Schematic representative of (a) 2-stage broken linear temperature response and (b) 2-stage broken linear photoperiod response (adapted from Birch et al. 1996, 1998a, Holzworth and Hammer 1992).
1.6 Steps in this research

Activities to meet the above objectives are described in following components of this thesis.

(i) A review of literature (Chapter 2) focusses on broccoli development, yield and quality, and the effects of temperature, photoperiod and solar radiation on development, yield and quality. Also, models developed for prediction of ontogeny were examined and the need for a simple and precise model emphasised.

(ii) Chapter 3 describes the site and experimental methods that are common to the experiments in Chapters 4 to 8 to avoid repetition.

(iii) Chapter 4 describes a reliable and repeatable method of detecting floral initiation based on a sequential series of scanning electron micrographs, supported by descriptions of the transition from vegetative to reproductive apex. Floral initiation was identified in a field experiment by comparing broccoli apices under a light microscope with standard electron micrographs of shoot apices.

(iv) The resistance of broccoli plants to extracellular freezing was tested in Chapter 5 by simulating natural frost events, where temperatures were reduced slowly in pot and field experiments.
(v) The necessary data were obtained from a field experiment to develop improved equations for head yield and quality (Chapter 6), and crop development (Chapter 7) responses to temperature and photoperiod. Development, yield and quality of 3 broccoli cultivars sown on 8 sowing dates from 11 March to 22 May 1997 under natural and extended (16 h) photoperiods, were studied at Gatton College. Also, thermal time models were successfully tested on independent data from five cultivars grown commercially in the Darling Downs, near Brookstead, over two years.

(vi) The thermal time models from emergence to floral initiation, from floral initiation to harvest maturity and from emergence to harvest maturity, developed in Chapters 7 and 8, are compared in Chapters 8 and 9. The emergence to harvest maturity thermal time model was also tested on data collected from Gatton in the early 1980s (Chapter 8). The relative merits of the models are discussed.
Chapter 2
Review of Literature

2.1 Introduction

For the development of models for prediction of ontogeny and quality of broccoli, a sound understanding of crop physiology and responses to environmental factors is essential. Models used for prediction of ontogeny and quality need to be robust and stable across environments and genotypes. Location or environment specific relationships need to be avoided (Birch 1996). This review will examine current literature on physiological responses of broccoli to major environmental factors, and the latest developments in models for the prediction of ontogeny and maturity.

The Literature Review has been divided into six main topic areas:
(a) Broccoli development;
(b) Techniques used for the evaluation of yield and quality of fresh market broccoli;
(c) Effect of temperature on ontogeny, yield and quality;
(d) Effect of sub-zero temperature injury on yield and quality;
(e) Effect of other environmental factors (photoperiod and solar radiation) on ontogeny, yield and quality; and
(f) Review of models developed for prediction of ontogeny and maturity.

2.2 Broccoli development

2.2.1. History and botany

Broccoli is the annual green heading form of *Brassica oleracea* L. var. *italica* Plenck grown in Australia, New Zealand, Japan, the United States of America, Canada, Germany, and The Netherlands since the late 1980s. The same green heading form of broccoli is known as calabrese (a derivation from Calabria in southern Italy) in the United Kingdom and Italy (Gray 1982, 1989, Titley 1985). Before the advent of
popular heading forms, the term broccoli was used to describe the green sprouting form (see Fig. 2.1) (Nieuwhof 1969, Seelig 1971, Tiley 1985). The green sprouting form produced most of its yield from multiple harvests of lateral heads or sprouts whereas the heading form produced a large, single, terminal inflorescence.

Broccoli is thought to have originated in the eastern Mediterranean. The crop was introduced into Italy where crop diversification took place and open pollinated, sprouting and coloured heading forms were developed. Broccoli was introduced to western Europe in the 18th century and north America in the 20th century (Gray 1982). Development of single heading hybrids by plant breeders in the United States, Japan, The Netherlands and United Kingdom during the 1970s revolutionised broccoli production in the 1980s and 1990s (Shinohara 1984, Tiley 1985).

Broccoli belongs to the family Brassicaceae, which includes other vegetable crops such as cauliflower (B. oleracea L. var. botrytis Alef), cabbage (B. oleracea L var. capitata Alef.), Brussels sprouts, kohl-rabi and kale. The distinction between broccoli and cauliflower can be made by their comparative morphology at the harvestable stage (Fig. 2.1). At harvest, the surface of the broccoli head is made up of a mass of fully differentiated floral buds whereas the cauliflower head (curd) is a dome of tissue made up of a mass of floral primordium meristems (Sadik 1962, Malatesta and Davey 1996). Marketable broccoli is ontogenetically more mature than marketable cauliflower. Most broccoli are green in colour due to chlorophyll within the sepals of the floral buds. This contrasts with the white or cream colour curd in cauliflower which lacks chlorophyll (Gray 1982, 1989, Shinohara 1984).
2.2.2. Phenological development and ontogeny

Phenology is the study of biotic events that occur once in a growing season of a crop (Alm et al. 1991). It describes, and measures developmental and physiological processes controlling growth and development, and the environmental influences. The following seven phenological stages of broccoli were described for crop protection purposes (i.e. control of weed, disease and insect pests) (Theunissen and Sins 1984):

(0) Seed stage - Spherical and brownish-black seed.

(1) Seedling stage - The seed has germinated and the lamina of the two cotyledons unfold at the top of the hypocotyl. The leaf-sheaths are still united.

(2) First leaf stage - The first true leaf develops between the fully extended cotyledons.
(3) Transplanting stage - The two first true leaves originate at the same height as the cotyledons. More leaves are formed and the plant grows.

(4) Vegetative stage - Axillary buds develop on the leaf sheaths. Older leaves show an axis of about 45° with soil level. Younger leaves stand upright.

(5) Harvesting stage - The inflorescence develops from the terminal apex and grows until it has reached its maximum size as a marketable product.

(6) Flowering stage - Flowering.

(7) Seed production stage - Seed development

Stages 6 and 7 are used mainly for plant breeding and seed production purposes, and are not directly relevant for fresh market broccoli production. Stages 3 and 5 are ‘human’ imposed stages that have limited biological meaning but are relevant for agronomic purposes. The floral initiation (FI) stage, essential for prediction of harvest dates (Wurr et al. 1991a), was not identified in the stages mentioned above.

A more practical distinction of phenological stages for the purpose of scheduling the harvest dates of fresh market broccoli was defined for the Lockyer Valley, Queensland (Fyffe and Titley 1989). Three phenological stages were described as follows:

Developmental stage 1 - Sowing to 50% emergence (SE)
Developmental stage 2 - 50% emergence to 50% floral initiation (EFI)
Developmental stage 3 - 50% floral initiation to 50% harvest maturity (FIHM)
(a) Developmental stage 1

Developmental stage 1 is the phenological interval from sowing to emergence and thus includes germination. Epigeal germination occurs in broccoli seedlings as cotyledons push above the soil surface, turn green, and function as leaves. Researchers working under controlled temperature conditions defined germination of broccoli as the protrusion of the radicle from the seedcoat, and emergence as 10 mm elongation of the radicle (Elson et al. 1992). Under field studies, it is more practical to define emergence as the penetration of the seedling hypocotyl through the soil surface (Heather and Sieczka 1991), which is the definition used throughout this thesis. If transplanted broccoli are used for crop establishment, developmental stage 1 occurs in the seedling nursery.

(b) Developmental stage 2

Developmental stage 2 is the phenological interval from emergence to FI. The FI stage (also known as head initiation) is not clearly defined in the literature. Chronological and morphological apex development of ‘Coastal’ broccoli during the transition from vegetative to reproductive tissue differentiation was studied (Gauss and Taylor 1969a). Histological methods on serial longitudinal sections of apex were used to relate morphological changes to leaf number and plant age (Table 2.1) and apex diameter at FI has been defined by several researchers (Table 2.2).

Table 2.1. Morphological changes in broccoli in relation to leaf number and plant age (adapted from Gauss and Taylor 1969a).

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Plant age (weeks)</th>
<th>Number of true leaves initiated</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative</td>
<td>0 - 4.5</td>
<td>0 – 6</td>
<td>Leaf primordium surrounds a small apex.</td>
</tr>
<tr>
<td>Transitional</td>
<td>4.5 - 6.5</td>
<td>6 – 11</td>
<td>Apex broadens and phenomenon of leaf cupping observed.</td>
</tr>
<tr>
<td>Floral initiation</td>
<td>6.5 - 9</td>
<td>11 – 22</td>
<td>First-order floral branch primordia visible.</td>
</tr>
</tbody>
</table>
Table 2.2. Broccoli apex diameter at floral initiation.

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Apex diameter (µm)</th>
<th>Experimental conditions</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative</td>
<td>100 - 147*</td>
<td>Field</td>
<td>Gauss and Taylor (1969a)</td>
</tr>
<tr>
<td></td>
<td>&lt;2000</td>
<td>Field</td>
<td>Fyffe and Titley (1989)</td>
</tr>
<tr>
<td></td>
<td>&lt;490</td>
<td>Controlled env.</td>
<td>Wurr et al. (1995)</td>
</tr>
<tr>
<td>Transitional</td>
<td>183 - 227*</td>
<td>Field</td>
<td>Gauss and Taylor (1969a)</td>
</tr>
<tr>
<td></td>
<td>&gt;2,000</td>
<td>Field</td>
<td>Fyffe and Titley (1989)</td>
</tr>
<tr>
<td>Floral initiation</td>
<td>259 - 275*</td>
<td>Field</td>
<td>Gauss and Taylor (1969a)</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>Controlled env.</td>
<td>Wiebe (1975)</td>
</tr>
<tr>
<td></td>
<td>&gt;2,000</td>
<td>Field</td>
<td>Fyffe and Titley (1989)</td>
</tr>
<tr>
<td></td>
<td>≥490</td>
<td>Controlled env.</td>
<td>Wurr et al. (1995)</td>
</tr>
</tbody>
</table>

* Gauss and Taylor (1969a) defined the width of the apex meristem as the horizontal distance between the 2 uppermost leaf or flower stalk primordia.

There is no consistency between the various reports of apex diameter at FI (Table 2.2), possibly because of variations in definition of each stage or different techniques used for viewing the shoot apex.

Several researchers (Fontes et al. 1967, Diputado and Nichols 1989) have attempted to define FI by dissecting apices under a light microscope and detecting differentiation of reproductive tissue. Japanese researchers (Fujime and Hirose 1979, 1980, 1981, Fujime et al. 1988) have used a nine-stage scale for broccoli and cauliflower but the scale lacked detailed description and illustration. An atlas of scanning electron micrographs of the FI stage in field crops in Australia (Moncur 1981), provides a set of standard photographs to identify transitional stages from vegetative to reproductive tissue differentiation in a range of crops, but not for broccoli. The advantages of electron micrographs are clarity of images and large depth of field. FI is defined as the stage when a new reproductive structure first develops on a previously vegetative apex (Salter 1969). A set of photographs is available for cauliflower (Moncur 1981) and has been used in research on broccoli reported in this thesis.
Developmental stage 3 is the phenological interval from FI through buttoning (or button formation) to harvest maturity (HM). The buttoning stage is defined as the stage at which the immature inflorescence (or button) head is approximately 10 mm in diameter (Gauss and Taylor 1969b, Marshall and Thompson 1987a). The buttoning stage was referred to as budding by Japanese researchers who studied the prediction of buttoning and harvest of broccoli (Fujime and Okuda 1994). Buttoning was identified by Deuter (1997) as a distinct stage for prediction of broccoli performance in south-east Queensland, during crop simulation studies using the expert system, Plantgro® (Hackett 1991).

HM of a broccoli head is primarily determined by the developmental stage of its florets. Broccoli is harvested at the optimum maturity stage, also referred to in the industry as ‘optimum harvest time’.

Due to non-uniformity in HM, most researchers have defined maturity date as the day on which 50% of the heads have reached maturity (Fyffe and Titley 1989, Marshall and Thompson 1987b, Diputado and Nichols 1989, Wurr et al. 1991a, Wurr et al. 1992). Immature heads were described as having ‘light green buds, which were tightly closed and held together compactly’ (Wurr et al. 1991a). Over-mature heads were defined in the Queensland Fruit and Vegetable Grading and Packing Regulations as ‘any head which contains flower buds which are open to the extent that the flower petals are visible’ (Sullivan 1979). Researchers in the United Kingdom (Pearson and Hadley 1988, Wurr et al. 1991a, 1992) have used specific broccoli head diameters (eg. 75 and 110 mm) as a measure of maturity, but actual size must depend on cultural and environmental conditions. Head diameter was measured in two directions at 90° to each other and averaged (Wurr et al. 1991a). In this thesis, HM is defined as the stage where 50% of broccoli heads have reached an inflorescence diameter of 100 mm (Dufault 1997, Grevsen 1998). This coincides with the developmental stage just before the buds start to open.
Although researchers working in Tasmania (Chung 1982, 1985a, Chung and Strickland 1986) reported the possibility of once-over harvest for processing broccoli, most cultivars do not mature uniformly and must be harvested selectively in a series of harvests. There was a maximum of 50% mature heads in any single harvest (Walton and Casada 1988). For fresh market broccoli, once-over harvesting is uncommon and not practical because it results in wide variation in size and quality.

2.3 Yield and quality of broccoli

Yield usually refers to marketable fresh weight of broccoli heads per unit area (t ha\(^{-1}\)) or mean head fresh weight (g head\(^{-1}\)). In the Australian broccoli industry, yield is often expressed as number of polystyrene cartons (usually 8 kg) per unit area.

2.3.1 Yield and harvest index

Various reports of yield and comparisons of yields across different experiments reflecting different cultural methods and different environments are listed in Table 2.3. For example, yield from sequential harvests ranged from 12.0 to 23.0 t ha\(^{-1}\) (Chung 1985b) compared with yield from once-over harvests that ranged from 5.7 to 10.5 t ha\(^{-1}\) (Chung 1985a). This yield difference reported by the same author (Chung 1985a, 1985b) in Tasmania was due to additional terminal and lateral heads harvested during multiple, sequential harvests.

Many workers also measured mean head fresh weights (g head\(^{-1}\)) as an indication of yield in plant density and cultivar experiments (Cutcliffe 1971, 1975, Thompson and Taylor 1976, Tittley 1981, Chung 1985a, 1985b, Chung and Strickland 1986, Mckay 1988, Lindsay and Zeppa 1990, Heisswolf and Deuter 1991). Yield per unit area can be calculated as a product of head fresh weight and plant population. Export quality broccoli for the south-east Asian market was described as having a mean head fresh weight of 275-350 g and stalk length of 150 mm (Tittley 1981).
Table 2.3. Reports of broccoli marketable head yield in studies covering a range of environmental and crop cultural conditions.

<table>
<thead>
<tr>
<th>Marketable yield (t ha(^{-1}))</th>
<th>Experimental conditions</th>
<th>Location</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.7 to 10.5</td>
<td>Field (once-over harvest)</td>
<td>Forth, Tas., Australia</td>
<td>Chung (1985a)</td>
</tr>
<tr>
<td></td>
<td>2.8 to 49.0 plants m(^{-2})</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Planted in December, January and March.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.0 to 23.0</td>
<td>Field (sequential harvest)</td>
<td>Forth, Tas., Australia</td>
<td>Chung (1985b)</td>
</tr>
<tr>
<td></td>
<td>2.8 to 16.7 plants m(^{-2})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.0 to 14.0</td>
<td>Field (once-over harvest)</td>
<td>Forth, Tas., Australia</td>
<td>Chung and Strickland (1986)</td>
</tr>
<tr>
<td></td>
<td>Planted from November to May. Cultivar trial.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.9</td>
<td>Field (sequential harvest)</td>
<td>Gatton, Qld., Australia</td>
<td>Titley (1987)</td>
</tr>
<tr>
<td></td>
<td>Planted from February to July. Cultivar trial.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0 to 11.2</td>
<td>Field (sequential harvest)</td>
<td>Gatton, Qld., Australia</td>
<td>Titley (1981)</td>
</tr>
<tr>
<td></td>
<td>Planted all year round. Cultivar trial.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.6 to 5.3</td>
<td>Field Cultivar trial.</td>
<td>Simcoe, Ontario, Canada</td>
<td>Shattuck et al. (1986)</td>
</tr>
<tr>
<td></td>
<td>Glasshouse</td>
<td>Netherlands</td>
<td>Mol (1985)</td>
</tr>
<tr>
<td>14.2</td>
<td>cv. Bravo, 8.2 plants m(^{-2})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.8</td>
<td>cv. Clipper, 16.6 plants m(^{-2})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.8</td>
<td>Field Single row, 6.5 plants m(^{-2})</td>
<td>Calhoun, USA</td>
<td>Lancaster et al. (1985)</td>
</tr>
<tr>
<td>12.4</td>
<td>Double row, 6.5 plants m(^{-2})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.6</td>
<td>Approx. av. yield (USA) USA</td>
<td>Lorenz and Maynard (1988)</td>
<td></td>
</tr>
<tr>
<td>13.4</td>
<td>Good yield (USA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.8 to 7.2</td>
<td>Approx. av. yield (Queensland) Qld., Australia</td>
<td>Heisswolf and Deuter (1992)</td>
<td></td>
</tr>
</tbody>
</table>

Higher plant weights and leaf areas were associated with higher broccoli head weights (Guan et al. 1995). Reducing irradiance by 1% resulted in a 0.9% decrease in dry
matter of broccoli plants at harvest and in a 1% decrease in head yield (Klaring 1998). In the closely related cauliflower, partitioning of dry matter to the curd depended on thermal time after FI and was described using a logistic growth function (Kage and Stutzel 1999). Total dry matter production rate was calculated using the product of intercepted photosynthetically active radiation and light use efficiency. The cauliflower curd started to become an important carbohydrate sink approximately 400 °C d (calculated using $T_{\text{base}}$ of 0 °C) after FI and approached its maximum fraction of total growth rate around 800 °C d after FI. At the end of the growing period, the rate of leaf dry matter production decreases, the growth of stem dry matter almost stops, and any increases in dry matter is attributable to head growth (Kage and Stutzel 1999).

At optimum maturity, broccoli heads were described as having been cut, trimmed to 150 mm in length and all bracts longer than 10 mm removed (Chung 1982). These bract-free and trimmed heads were recorded as marketable yield. All other heads (under-mature, over-mature or non-productive) in addition to the bracts and extra stem lengths of the marketable heads were weighed separately and recorded as vegetation. The fresh weight harvest index (FWHI) (Chung 1982, Shelp 1988, Jett et al. 1995) was defined as follows:

\[ \text{FWHI} = \frac{100 \times \text{head fresh weight}}{\text{head fresh weight} + \text{vegetation fresh weight}} \]  

2.3.2 Quality

A key to rating the quality attributes of head size, head shape, branching angle, bud size, and physiological disorders of broccoli was developed in the UK (Chowings 1974) and modified for Tasmanian conditions (Chung 1982). Most Australian researchers have used the modified key (Chung 1982) for quality evaluations (Chung 1985a, Titley 1981, 1985, 1987, Rettke 1990).

Few researchers have studied the effect of environmental and agronomic conditions on quality attributes of broccoli. This is probably due to difficulty in measuring and
rating subjective quality attributes. Most researchers have used univariate methods to assess quality. A multivariate approach using principal components analysis was used to assess the quality of crisphead lettuce cultivars growing under hydroponic conditions in Queensland (Tan 1991). This approach may hold promise for assessing broccoli quality.

The Australian Fruits and Vegetables Standards for broccoli (Section 8.2) was drafted in 1984 but has yet to be adopted by industry (Milgate, M. 1996, pers. comm.). A product description for broccoli was developed for the Australian United Fresh Fruit & Vegetable Association mainly for the purpose of post-harvest handling and marketing (Story and Martin 1996). A detailed quality scale was developed specifically for post-harvest assessment of broccoli quality at the Institute for Horticultural Development, Knoxfield, Victoria (O’Donnell et al. 1996), in which 14 characteristics of the broccoli head were assessed and variations of each individual trait presented as a series of colour photographs. Some quality attributes are discussed in greater detail below.

(a) Bractiness

Bractiness refers to bracts throughout the head and possibly emerging through florets and thus appearing above the top of the head (Story and Martin 1996, Dufault 1996). It is a typical physiological symptom of heat stress (Heather et al. 1992). Bracts developed rapidly when the inflorescence was subjected to high temperatures at the time of flowering (Haine 1951).

(b) Hollow stem

The earliest signs of this disorder are small elliptical cracks developing in the inner stem tissue. As plants approach maturity, these cracks may enlarge and coalesce, causing the stem to become hollow (Shattuck et al. 1986). The decreased incidence of hollow stem was associated with reduced plant density (Cutcliffe 1972, Griffith and Carling 1991) and could be affected by cultivar (Cutcliffe 1975, Shattuck et al. 1986). The incidence of hollow stem increased with boron deficiency (Shattuck and

(c) Starring

Starring refers to uneven bead size in the florets, giving a star-like appearance within the head (Story and Martin 1996).

(d) Albugo candida (White Blister)

Affected buds are white and grow much larger than healthy buds (Thompson and Taylor 1976).

(e) Colour

Most of the work on colour assessments of broccoli was done in post-harvest storage studies. Broccoli colour has been evaluated using a subjective scale (Lipton and Harris 1974). The major limitation of past colorimetric studies has been inability of instruments to compensate for head curvature. However, newer generation colorimeters (e.g. Gardner XL-845) were reported to provide a rapid, non-destructive alternative to chlorophyll analysis in the objective measurement of broccoli colour (Shewfelt et al. 1984). In Australia, broccoli floret colour has been measured non-destructively by reflectance using a colour meter (Chroma meter II) (King and Morris 1994a, 1994b).

This section described broccoli yield, harvest index and quality. The next section (Section 2.4.1) will describe the effect of temperature on broccoli development and quality.

2.4 Effect of temperature

2.4.1 Cardinal temperatures for development
Cardinal temperatures are the base, optimum and maximum temperatures ($T_{\text{base}}$, $T_{\text{opt}}$ and $T_{\text{max}}$) used for calculating thermal time (TT). These temperatures refer to the temperatures at and below which crop development ceases ($T_{\text{base}}$), (and no TT is accumulated), crop development proceeds at the maximum rate ($T_{\text{opt}}$), and above which crop development ceases ($T_{\text{max}}$) (Birch 1996). Terms referring to TT include degree-days, growth units, heat sums, heat unit accumulation and heat summation. In this thesis, the terms TT and degree-days ($^\circ$C d) will be used for consistency.

Most research has been directed to definition of $T_{\text{base}}$ and much less to determination of $T_{\text{opt}}$ and $T_{\text{max}}$ (Table 2.4). There is also some discrepancy in calculation of $T_{\text{base}}$ between researchers. For example, for the same cultivar ‘Futura’, Chung (1981) reported a $T_{\text{base}}$ of -4 °C whereas Titley (1985) reported a $T_{\text{base}}$ of 1 °C. This is probably due to differences in methods used to calculate $T_{\text{base}}$. Chung (1981) used the linear model of Arnold (1959) which resulted in a much lower $T_{\text{base}}$, whereas Titley (1985) used the non-linear, rectangular hyperbola model of Mead et al. (1993).

The temperatures mentioned above are air temperatures but within a plant, temperatures may vary. Researchers in Japan have observed under field conditions that broccoli shoot tip temperature became lower than the surrounding air temperature at night and equal to or a little higher than the air temperature during the day as the amount of irradiance increased. The stem temperature was generally higher than the temperature of leaf and shoot (Fujime and Hirose 1984).

Table 2.4. Base, optimum and maximum temperatures for the development of broccoli.

<table>
<thead>
<tr>
<th>Phenological interval</th>
<th>Cultivar</th>
<th>$T_{\text{base}}$ ($^\circ$C)</th>
<th>$T_{\text{opt}}$ ($^\circ$C)</th>
<th>$T_{\text{max}}$ ($^\circ$C)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cultivar</td>
<td>Average Growth</td>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>---------------</td>
<td>-----------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Futura’</td>
<td>-4</td>
<td>Chung (1981)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Gem’</td>
<td>-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Remco’</td>
<td>-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Yates 12’ (Grp A)</td>
<td>6.8</td>
<td>Titley (1985)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Selection 160’ (Grp B)</td>
<td>5.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Selection 165A’ (Grp C)</td>
<td>5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Premium Crop’ (Grp D)</td>
<td>6.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Futura’</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Corvet’</td>
<td>0</td>
<td>Marshall and Thompson (1987a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Mercedes’</td>
<td>7</td>
<td>Pearson and Hadley (1988)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Corvet’</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Premium Crop’</td>
<td>1</td>
<td>Diputado and Nichols (1989)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Mercedes’, ‘Idol’</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Fordhook Late’</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Citation’, ‘Prima’, ‘Cruiser’, ‘Corvet’, ‘Skiff’</td>
<td>0</td>
<td>Wurr et al. (1991a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Baccus’, ‘Citation’, ‘Packman’, ‘Southern Comet’</td>
<td>7.2</td>
<td>Dufault (1997)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Caravel’, ‘Shogun’, ‘Emperor’</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 2.4.2 Effect of temperature on phenological development

(a) **Seed vernalisation**

Seeds of broccoli cultivar ‘Coastal’ were vernalised by soaking them in water (in the dark) at room temperature for 24 h and stored in the refrigerator (in the dark) for 30 days at -0.5 °C (Gauss and Taylor 1969b). Seed vernalisation (low temperature promotion of flowering) did not result in any chronological or developmental advancement of the apex toward FI, which implied that ‘Coastal’ did not have any seed vernalisation requirement.
However in Japan, early cultivars grown from vernalised seed showed slight acceleration of FI and flowering compared to non-vernalised controls (Kagawa 1965). Response to seed vernalisation was not observed in late cultivars. Total leaf number and stem length at HM slightly decreased as duration of seed vernalisation at 0 °C was increased from 30 to 45 days (Fujime and Hirose 1979). Terminal head weight and diameter, and number of lateral heads per plant, were increased with the longer treatment.

(b) Developmental stage 1

Temperatures experienced during developmental stage 1 (sowing to emergence) can affect the rate of germination and emergence. Reported cardinal temperatures for the germination of broccoli are listed in Table 2.5.

Table 2.5. Base, optimum and maximum temperatures for germination of broccoli.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>$T_{base}$ (°C)</th>
<th>$T_{opt}$ (°C)</th>
<th>$T_{max}$ (°C)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Selection 160’</td>
<td>5</td>
<td>20/20</td>
<td>30</td>
<td>Heslehurst (1984)</td>
</tr>
<tr>
<td>(Day/night)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Packman’</td>
<td>&lt;5</td>
<td>Not available</td>
<td>&gt;30</td>
<td>Elson et al. (1992)</td>
</tr>
</tbody>
</table>

In constant temperature growth cabinets, greater than 90% germination was achieved for broccoli cultivar ‘Packman’ for the range of temperatures between 5 °C and 30 °C (Elson 1992). Even though 5 °C was sufficient for 90% germination, growth rate was so slow that radicles did not reach 10 mm for 14 days. Time to emergence decreased at a linear rate as mean soil temperatures increased from 6 °C to 24 °C for cultivar ‘Rex’ (Hegarty 1978). At higher temperatures, germination and radicle emergence of cultivar ‘Packman’ decreased at a linear rate as air temperatures increased from 30 °C to 38 °C. High temperatures limited radicle growth more than germination (Elson et al. 1992).
(c) Developmental stage 2

Timing of FI in developmental stage 2 (emergence to FI) and flowering may be influenced by vernalisation. However, some of the differences in timing of FI are probably responses to higher temperatures rather than vernalisation. Cultivar ‘Coastal’ grown at both 13 °C and 29 °C formed buttons (see Section 2.2.2.c) at the same chronological time (74 days from the sowing) (Gauss and Taylor 1969b). However, temperature differences affected development at buttoning with regard to the number of leaves initiated. At 29 °C, 27 leaves were initiated compared with 17 leaves when plants were grown at 13 °C. The authors thought that the cultivar, ‘Coastal’ did not have any vernalisation requirement for FI. The same cultivar was believed to have a quantitative vernalisation effect because leaf number was increased from 18 to 24 as temperature increased from 13 °C to 29 °C (Wiebe 1975). However, differences in leaf number only suggests a difference in development and do not prove whether ‘Coastal’ has any vernalisation requirement. Seedlings of cultivars ‘Gem’ and ‘Bravo’ that were 13 or more days from sowing and exposed to 14 or more days of low temperatures (-5 °C to 2 °C) flowered earlier and had fewer nodes than plants not exposed to low temperatures (Miller et al. 1985). Leaf expansion rate during the vegetative stage was unaffected by radiation and fitted a broken linear growth model with $T_{\text{base}}$ of –0.7 °C and $T_{\text{opt}}$ of 21 °C (Olesen and Grevsen 1997).

(i) Beginning of receptiveness

The juvenile phase is defined as ‘the interval between the germination of a seed and the ability of the seedling to respond to an environmental signal inducing flowering’ (Friend 1985). The literature suggests the existence of a juvenile phase in broccoli (Kagawa 1965, Fontes et al. 1967, Miller et al. 1985, Fujime 1988, Wiebe 1990). Cold treatment for 3 weeks at 4.4 °C induced floral primordia development in all 5 week old and some 4 week old broccoli plants at the beginning of treatment but not in 3 week old plants (Fontes et al. 1967). Duration of the juvenile phase in several cultivars is tabulated in Table 2.6. Receptiveness (i.e. ability to respond to an environmental signal inducing flowering) to induction may be influenced by genetic
differences between cultivars (Fujime 1988). Site of perception of low temperature in cabbage, which is closely related to broccoli, was the stem apex (Friend 1985).

The model of vernalisation (Wurr et al. 1995) developed to predict change in apex diameter of broccoli with temperature did not include a juvenile phase. It is based on data from seedlings that were transplanted at the 8-leaf stage. The juvenile stage may have already been passed before transplanting. The authors commented that exponential change in apex diameter still implies that plants must grow for several weeks before apex diameter increases sufficiently for floral induction. They argued that plants must still produce a minimum plant weight, stem diameter and number of leaves before responding significantly to vernalising temperatures. Critical stem diameter of plants that were receptive to low temperatures was 5-8 mm which corresponded with plant fresh weight ranging from 4 g to 50 g, the upper limit of the receptive stage (Miller et al. 1985). This suggests that there is no precise plant weight at which change to a reproductive apex occurs. A slightly smaller critical stem diameter of 3.5 mm and expanded leaf number of 5 or 6 was required for broccoli cultivar ‘De Cicco’ to be responsive (Kagawa 1965). The end of the juvenile stage was defined as when a broccoli plant has more than 4 leaves greater than 20 mm in length (Wiebe 1990). The concept of juvenility measured in numbers of leaves did not apply consistently in field-grown cauliflower plants and measurement of apex diameter to estimate the end of juvenility, required tedious microscope techniques (Fellows et al. 1999). The juvenile stage in the closely related cauliflower was thought to end at an apex diameter of approximately 0.2 mm (Wurr and Fellows 1998). Juvenile stages of cauliflower cultivars, ‘Perfection’ and ‘Gypsy’ were estimated to end at apex diameters of 0.25 and 0.27 mm, respectively (Fellows et al. 1999).

Table 2.6. Effect of vernalisation (inductive temperature range and exposure time) on floral initiation of broccoli.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Cold requirement</th>
<th>Beginning of receptiveness</th>
<th>Inductive temp. range (°C)</th>
<th>Exposure time (weeks)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘De Cicco’ Early &amp; Medium</td>
<td>Not reported</td>
<td>Stem diameter at</td>
<td>5 – 22</td>
<td>3</td>
<td>Kagawa (1965)</td>
</tr>
<tr>
<td>cvs.</td>
<td>3.5 mm and leaf number of 5 or 6.</td>
<td>2 – 3</td>
<td>4.4 - 21</td>
<td>3</td>
<td>Fontes et al. (1967)</td>
</tr>
<tr>
<td>--------------</td>
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<td>----------------</td>
<td>----------</td>
<td>---</td>
<td>----------------------</td>
</tr>
<tr>
<td>‘Waltham 29’ ‘Green Mountain’ Facultative? 5 weeks after germination</td>
<td>T&lt;sub&gt;max&lt;/sub&gt; = 24</td>
<td>Gauss and Taylor (1969b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Coastal’ No effect except under continuous lighting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wiebe (1975)</td>
</tr>
<tr>
<td>‘Coastal’ Low facultative?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Gem’ ‘Bravo’ Not reported Critical stem size at 5 - 8 mm diam. Plant fresh wt. at 4 - 50 g</td>
<td></td>
<td>Miller (1985)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facultative Plants with more than 4 leaves (&gt; 20 mm)</td>
<td>T&lt;sub&gt;base&lt;/sub&gt; = 0 T&lt;sub&gt;opt&lt;/sub&gt; = 5 T&lt;sub&gt;max&lt;/sub&gt; = 20</td>
<td>2 - 4</td>
<td>Wiebe (1990)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Shogun’ Obligate</td>
<td>T&lt;sub&gt;base&lt;/sub&gt; = -2.8 T&lt;sub&gt;opt&lt;/sub&gt; = 15.8 T&lt;sub&gt;max&lt;/sub&gt; = 23.6</td>
<td>3 - 6</td>
<td>Wurr et al. (1995)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(ii) Vernalisation requirement

Some brassicas such as cabbage have an obligate (or qualitative) vernalisation requirement which means that they will stay vegetative for a number of years when grown continuously at high temperatures. Other varieties have a facultative (or quantitative) vernalisation requirement and will eventually flower at high temperatures although flowering is accelerated by low temperature (Friend 1985, Wiebe 1990). Evidence of an obligate vernalisation requirement in broccoli was apparent when plants grown continuously at 20.7 °C and 22.6 °C produced apices which were only 0.26 and 0.20 mm in diameter, and still remained vegetative after 10 weeks at those temperatures (Wurr et al. 1995).

(iii) Inductive temperature range
The base ($T_{\text{base}}$), optimum ($T_{\text{opt}}$) and maximum ($T_{\text{max}}$) temperatures for vernalisation in broccoli were reported to be 0, 5 and 20 °C respectively, but no data was presented (Wiebe 1990). The vernalisation model developed for broccoli used cardinal temperatures of -2.8, 15.8 and 23.6 °C (Wurr et al. 1995). However, these cardinal temperatures were estimated by extrapolation beyond the experimental data range of 7.3 to 22.6 °C and should be interpreted cautiously. In fact, plants grown at 20.7 and 22.6 °C did not initiate floral primordia and still remained vegetative at those temperatures, which suggests that $T_{\text{max}}$ is likely to be closer to 20 °C. Several authors have also reported that there may be a different inductive temperature range depending on the earliness of the cultivar (Kagawa 1965, Hackett and Carolane 1982, Shinohara 1984).

(iv) Premature floral initiation and flowering

Premature floral initiation refers to the timing of FI which has occurred too early, resulting in the production of a small head that is uneconomic (Baggett and Mack 1970, Wien and Wurr 1997). This phenomenon was thought to be due to low temperatures during late autumn sowings in Tasmania, Australia, which induce FI at a younger physiological age (Chung and Strickland 1986). Consequently, plants would develop heads before reaching full potential size, and heads would be small and take longer to reach maturity (Chung and Strickland 1986).

Premature flowering or bolting (rapid elongation of flowering shoots) is characterised by the yellow petals emerging from floral buds of broccoli inflorescences, before the heads have reached the required diameter or size for harvest, making them unmarketable. Premature flowering was achieved by chilling 14 day old seedlings for 14 to 28 days at 2 °C (Miller et al. 1985, Miller 1988). Broccoli seedling producers should avoid cold conditions during transplant production.

Although much of the literature used to describe developmental stage 2 and prediction of FI was based on vernalisation studies, many of the responses described were
temperature responses of leaf number and plant development. Hence, it may be possible to predict FI using thermal time models.

(d) Developmental stage 3

Broccoli is very sensitive to high temperatures during developmental stage 3 (FI to HM) (Heather et al. 1992, Bjorkman and Pearson 1995, 1998). Field and greenhouse studies showed that heat stress (1 week at 35 °C) may be most critical at 3 weeks before harvest for broccoli cultivar ‘NVH 521’, corresponding to the time the immature inflorescence measures 5 to 10 mm (buttoning stage) in diameter (Heather et al. 1992). The greatest limitation on broccoli production in summer at Goondiwindi (Queensland) appears to be heat damage during developmental stage 3, based on simulations using the Plantgro® model (Deuter 1997).

However in broccoli cultivar ‘Galaxy’, the developmental stage most sensitive to heat (1 week at 35 °C) was much earlier, at FI (Bjorkman and Pearson 1995, 1998). The injury was a cessation of bud enlargement during high temperature exposure, and there was no corresponding cessation of bud initiation at the apex. The most sensitive stage was when the apex diameter was less than 1 mm wide and floral primordia were just forming, still subtended by bract primordia. After buds had differentiated, they were no longer sensitive. Only approximately one third of the buds were affected and the injury was fully expressed when the head was 10 mm (buttoning stage). Affected buds that were arrested in development by high temperature, developed into fertile flowers about a week later than the unaffected buds (Carr and Irish 1997). The size contrast between the delayed buds and unaffected buds caused unevenness in the head.

2.4.3 Effect of temperature on quality

Effect of temperature on head development for broccoli and cauliflower has been studied in Japan (Fujime 1983, Fujime and Okuda 1996). Head abnormalities included descriptions such as ‘blindness’, ‘fuzziness’, ‘bractiness’ and ‘riciness’. Blindness is the death of the growing point and occurs at a low incidence in brassicas.
Studies in the UK showed high levels of blindness in broccoli cultivar ‘Marathon’ were strongly associated with low solar radiation, rather than with low temperatures (Wurr et al. 1996b). The other head abnormalities, fuzziness and riciness are mostly found in cauliflower (Fujime and Okuda 1996), and bractiness is described in Section 2.4.3.b.

Responses of broccoli cultivars to growing season mean (GSM) temperatures for several important market quality characteristics, such as head shape, colour, cluster separation, bractiness, and bud size were studied by Dufault (1996). GSM temperature was defined as the daily minimum and maximum temperatures from transplanting to last harvest for each sowing date and cultivar. Regression analysis techniques were used to determine which GSM temperature variable (minimum or maximum) contributed most to each quality variable. The acceptable GSM temperature range of performance for each quality variable was defined as the point where ≥ 85% of the population were classified as acceptable. GSM minimum or maximum temperatures did not affect bud size of any cultivar for any sowing date studied (Dufault 1996). A summary of the GSM temperature range at which broccoli head quality is unacceptable is given in Table 2.7.

Table 2.7. Growing season mean temperatures (°C)* at which broccoli head quality is unacceptable for the range of temperatures experienced during 50 sowing dates at Charleston, S.C., USA, from 1990 to 1992 (adapted from Dufault 1996).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Colour</th>
<th>Bractiness</th>
<th>Cluster separation</th>
<th>Head shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Baccus’</td>
<td>&lt;20.3 max</td>
<td>---***</td>
<td>---***</td>
<td>&lt;19.8 and &gt;26.8 max</td>
</tr>
</tbody>
</table>
* Temperatures derived from regression analysis. Mean minimum and maximum temperatures during the broccoli production seasons ranged from about 7.0 to 23.5 °C and 18.5 to 32.5 °C, respectively.
** Greater than 85% of all heads remained acceptable within the range of temperatures experienced in the field.

\[
\begin{array}{|c|c|c|c|c|}
\hline
\text{Variety} & \text{<21.0} & \text{>27.3 max} & \text{>20.2 max} & \text{<19.2} & \text{>28.9 max} \\
\hline
\text{Citation} & \text{max} & \text{<18.4} & \text{max} & \text{>25.7 max} \\
\hline
\text{Packman} & \text{large} & \text{>32.0 max} & \text{<8.4} & \text{max} & \text{>18.0 min} & \text{<22.0 max} \\
\hline
\text{Southern Comet} & \text{large} & \text{<9.2} & \text{and} & \text{>16.5 min} & \text{<32.0 max} & \text{<8.9} & \text{and} & \text{>16.2 min} & \text{<21.0} & \text{and} & \text{>25.3 max} \\
\hline
\end{array}
\]

\text{a) Effect of low temperature on quality}

The effect of low temperature on quality is not well documented in broccoli. Sub-zero temperatures may result in freezing injury and unmarketable broccoli heads (Section 2.5).

\text{b) Effect of high temperature on quality}

The effects of high temperature on quality were discussed in Section 2.4.2.d. The four reported physiological symptoms of heat stress in broccoli are:

1) Rapid swelling or puffing of sepals causing unevenness in the head due to widely differing sizes of buds (Heather et al. 1992, Bjorkman and Pearson 1995);
2) Elongation of the branchlets (peduncles) and irregular branchlet cluster separation (Heather et al. 1992);
3) Bractiness (leafiness) or protrusion of leaves through the head (Haine 1951, Heather et al. 1992, Dufault 1996, Fujime and Okuda 1996); and
4) Brown bead (Titley 1987).

This section described the influence of temperature on broccoli development and quality. The next section (Section 2.5) will describe crop injury caused by sub-zero temperatures.
2.5 Effect of sub-zero temperatures

Most low temperature work on broccoli studied vernalisation (Wiebe 1975, Wurr et al. 1995) or premature flowering (Miller et al. 1985, Miller 1988). Little work has been done on freezing injury. Thus, this section examines relevant work on freezing of other brassicas such as cauliflower, cabbage, and brassica oilseeds (B. campestris, B. napus, B. rapa and B. juncea). Reference will also be made to some other species.

2.5.1 Frost

(a) Radiation frost

Radiation frost is the main cause of freezing damage to crop plants in Australia. In south-east Queensland, damaging frosts occur during the night in conditions where there is inflow of cold air from the south or south west, very low humidity, and clear skies for rapid radiation of surface heat (Woodruff et al. 1997).

Heat absorbed during the day is lost from the ground surface and crop canopies at night as long wave radiation. When skies are clear and the air is cool and dry, most of this radiation is lost to space resulting in a rapid fall in ground and crop temperatures. Air in contact with these cooling surfaces becomes chilled and flows into lower lying areas, since it is denser. Movement of air can cause mixing of cold air near the ground surface with warmer air above reducing the severity of frost. If there is restricted or no air movement, severity of freezing can be greater since the minimum temperature reached will be lower (Lewis et al. 1989).

A sparse crop will allow radiation from the soil to reach the tops of the growing crop and allow the cold air in contact with these crop tops, which are rapidly radiating heat, to drain away and be replaced by warmer air (Woodruff et al. 1997). A temperature gradient builds up within a tall standing crop with a dense canopy. Heat is rapidly lost from the radiating canopy but not from the ground surface, since the crop intercepts this radiation. Heat is also conducted to the soil surface from deeper
within the soil. Hence, damage can be very variable since the minimum temperature will vary at different heights within the crop. Temperatures near the crop surface may be up to 2 °C colder than those in the middle of the crop canopy (Marcellos and Single 1975, Lewis et al. 1989).

As air temperature falls, dew point is reached. When initial water vapour content is high, dew appears on radiating surfaces. Fog occurs when the air becomes saturated with water vapour. If the air is very dry, ice may form directly on cold surfaces by sublimation. A microscopic nucleating agent needs to be present for dew to commence freezing. Such nuclei may be present on the leaves in the form of dust particles or epiphytic ice nucleation active bacteria such as Pseudomonas syringae and Erwinia herbicola (Lindow 1983). As ice forms, some heat is released (latent heat of fusion) and temperatures stop falling for some time. In this way, a hoar frost with visible ice formation occurs.

(b) Black frost

A black frost occurs under very low relative humidity conditions where the dew point of the air is lower than 0 °C. Very little surface ice is produced and, hence, a lower minimum temperature is reached since temperatures keep falling with little latent heat of fusion being released (Lewis et al. 1989, Perry 1998).

(c) Advection frost

Advection frost is caused by movement of a large mass of freezing air from polar regions. Such frosts are accompanied by windy conditions. They are not likely to be experienced in south-east Queensland (Lewis et al. 1989, Perry 1998).

2.5.2 Freezing injury

Freezing stress is a function of the freezing point of plant tissue at some temperature below 0 °C. Two types of freezing injury affect plants: primary direct freezing injury
caused by intracellular freezing; and secondary freezing injury, including freeze-dehydration, caused by intercellular freezing (Levitt 1980).

(a) Intracellular freezing

Intracellular freezing occurs when ice crystals form throughout the protoplast and vacuoles within the cell plasma membrane (Dhawan 1993). Intracellular ice formation is brought about by rapid freezing, or non-equilibrium freezing upon supercooling to well below the freezing point of the tissue. Ice crystal formation is then induced or occurs after a period of time. Cells in which ice crystals are formed are nearly always killed. Ice crystals damage protoplasmic structure, perhaps by lacerating membranes and destroying their semi-permeability (Levitt 1980). Further degradation may be caused by enzymes released due to breakdown of cellular compartmentalisation (Dhawan 1993). However, primary direct freezing injury caused by intracellular freezing has rarely been observed in nature (Levitt 1980). This phenomenon is mostly been observed during rapid (more than 1-2 °C h⁻¹ temperature reduction) artificial freezing.

(b) Intercellular freezing

In higher plants, the major form of freezing injury is secondary water stress associated with freeze-dehydration driven by extracellular ice formation. Under natural conditions, ice crystallisation normally begins in large xylem vessels, since dilute sap in these vessels has a high freezing point compared with cell sap in other plant parts. Once initiated, ice crystal formation spreads readily throughout the plant in those parts external to living cells. This is because the cell plasma membrane prevents ice crystals from inoculating the cell contents (Levitt 1980, Dhawan 1993). Growth of ice crystals in intercellular spaces drives outward movement of water from cells because of the water potential gradient that establishes. As a consequence, cell contents become more concentrated, resulting in freezing point depression and further
mitigation against the possibility of intracellular ice formation. The extracellular ice crystals may grow in mass to be larger than the cells. Thus, the plant cells may contract and even collapse. Desiccation of the protoplasm can damage cell organelles and cellular ultrastructure, and disrupt biochemical reactions (Levitt 1980).

During extracellular freezing, cells contract and air in the intercellular space can be driven out of the plant tissue resulting in a translucent appearance of the frozen tissue (Li 1984). Upon thawing, the tissues are limp and have a water-soaked appearance. When ice in the intercellular spaces is replaced by water from melting ice crystals, the spaces are essentially devoid of air. Cells are flaccid due to their loss of water to the ice masses when the ice masses were growing.

If the tissues are uninjured, intercellular water is soon re-absorbed by the cells. As they regain their turgor, air enters the extracellular spaces and the water-soaked appearance is quickly lost. Physically injured cells are however, unable to re-absorb water. Upon thawing, freeze-killed cells characteristically show plasmolysis, whereby a contraction of the dead protoplast leaves a large space between it and its cell wall. Plasmolysis is due to cell wall and protoplast contraction during extracellular ice formation, as well as the inability of dead, freely permeable protoplasts to re-absorb water formed in the intercellular spaces upon thawing of extracellular ice. That is, the elastic cell wall expands back to nearly its original shape, while the dead protoplast remains contracted, giving a false appearance of plasmolysis (Levitt 1980, Li 1984).

2.5.3 Resistance to freezing injury

Freezing resistance is the ability of an organism to survive the direct effects of freezing temperature without suffering permanent damage to its growth or reproduction. Freezing resistance of a plant results from two types of mechanisms - freezing avoidance, and freezing tolerance. Freezing avoidance involves delaying or preventing the penetration of the stress into tissues, thereby avoiding ice formation (Levitt 1980, Dhawan 1993). Freezing tolerance imparts an ability of the plant to
tolerate freezing, despite penetration of stress into the tissues and consequent intercellular freezing (Dhawan 1993).

(a) Freezing avoidance

Plants are unable to maintain a constant tissue temperature different from that of their environment. Therefore, they have no effective defence against low temperature and cannot possess a true capacity to avoid low temperature.

(i) By anti-freeze or dehydration

Cells may accumulate anti-freeze compounds, such as soluble anti-freeze proteins, to maintain their freezing point below that of their environment (Li 1984, McKersie and Leshem 1994). This ability may provide partial freezing avoidance due to accumulation of solutes in combination with dehydration. Dehydrated cell sap, as in seeds and pollen, has little freezable water. This option is only available to plants or plant parts with high water stress tolerance (Levitt 1980).

(ii) Supercooling

Plant sap seldom freezes at the freezing point of tissue water. Sap supercools to a few degrees below this point because of the lack of ice nucleating sites. Many woody species have exploited this phenomenon as a primary strategy to avoid freezing. However, protection cannot exceed the homogenous nucleation temperature of water, which is approximately –40 °C (McKersie and Leshem 1994).

Most plants freeze when their tissue temperature drops 1 - 2 °C below their freezing point because of the presence of nucleators either within the plant (e.g. xylem vessels) or on its surface (e.g. hoar frost). Factors that favour supercooling are small cell size, low moisture content, little or no intercellular space for nucleation, absence of internal
nucleators, barriers against external nucleators, and the presence of anti-nucleators (Levitt 1980).

(iii) Thermal insulation by wrapper-leaves

Curd tissues of cauliflower are less tolerant to freezing temperatures than are leaves (Grout et al. 1982, Fuller et al. 1994). The inner, wrapper-leaves of cauliflower and their enclosed air spaces provide significant protection for the curd tissues in mild frost, but are ineffective under severe conditions of below −2.5 °C. Once the curd surface is exposed, the thermal insulating effect of wrapper-leaves is lost (Tapsell et al. 1990).

(b) Freezing tolerance

The only form of freezing tolerance developed by plants is tolerance of the secondary water stress associated with extracellular ice formation. This tolerance is achieved by avoidance and tolerance of freeze-induced dehydration strain (Levitt 1980). Direct calorimetric measurements of ice formation in cabbage plants showed that hardening can involve both avoidance and tolerance strategies (Levitt 1980). The hardening process involved a large increase in avoidance of dehydration strain from −2.1 °C (unhardened) to −5.6 °C (hardened). There was also a very definite increase in tolerance of freeze-induced dehydration strain, from 60% of water frozen at the frost-killing temperature to 75% in hardened plants (Levitt 1939).

2.5.4 Cold acclimation (hardening)

The frost-killing point, although determined under standard conditions, is not a constant, even for a genetically pure strain. Rather, it varies markedly with the stage of development and with environmental factors that can alter resistance. Cold acclimation or hardening is a term describing the transition of a plant from a relatively frost-sensitive state to a relatively frost-tolerant state (Levitt 1980).

(a) Physiological factors during cold acclimation
Most studies have attempted to correlate acclimation, or developmental changes in tolerance, with metabolic changes. The following are general changes or effects that are often observed:

(i) Osmotic concentration

The major changes in osmotic potential are due to changes in sugars. The soluble sugars, sucrose, glucose and fructose gradually increased in cabbage leaves during cold acclimation, and their levels were positively correlated with the degree of freezing tolerance (Sasaki et al. 1996, 1998). Sugars may depress the freezing point of tissue, act as a nutrient and energy reserve, alter phase properties of membranes in the dry state, and act as a cryoprotectant to preserve protein structure and function (McKersie and Leshem 1994).

(ii) Water content

The water content of a tissue is inversely related to freezing tolerance. Water stress induces cold hardiness in cabbage (Cox and Levitt 1976, Sasaki et al. 1998). Acclimation promotes water loss from tissue, the presence of bound water (i.e. water held tightly in an unfreezable form), and accumulation of starch and protein which are not osmotically active (Levitt 1939). For example, during cold acclimation, dry matter in winter cereals accumulates at a faster rate than water, resulting in a decreased percent water content. Decrease in total water content could also be due to a loss of water in extracellular spaces (Li 1984).

(iii) Lipids
Total lipids, phospholipids, and phosphatidylcholine tend to accumulate in oilseed brassica (*B. napus*) leaf tissue during cold acclimation (Itzhaki *et al.* 1991). In many cases, the increase in lipids is associated with proliferation of cellular membranes (McKersie and Leshem 1994).

(iv) Proteins

There is a close correlation between soluble protein content and freezing tolerance. This elevated protein content is associated with an increase in the amount of messenger and transfer RNA required for protein synthesis (McKersie and Leshem 1994). An increase in total RNA was associated with increased freezing tolerance in *Brassica* spp. (Laroche *et al.* 1992).

(v) Growth regulators

The growth promoting effect of gibberellins is negatively associated with freezing tolerance, whereas dormancy caused by abscisic acid (ABA) is positively associated with tolerance (McKersie and Leshem 1994). During cold acclimation, ABA levels increase in oilseed brassica (*B. napus* and *B. campestris*) (Wilen *et al.* 1994) and gibberellins decrease (McKersie and Leshem 1994).

(vi) Photosynthesis

Low temperature acclimation of seedlings and plants requires photosynthesis, and hence light and carbon dioxide. These factors presumably support accumulation of sugars, proteins and solutes. However, the relationship between photosynthesis and tolerance is complex, depending upon the species and plant stage of development (Levitt 1980, McKersie and Leshem 1984).

White inner wrapper-leaves of cauliflower appeared to be more sensitive to frost than green fully mature leaves (Fuller *et al.* 1989). Further, white cauliflower curds were damaged at a higher temperature (-2.8 °C) than green curds (-6.0 °C) (Grout *et al.* 1982). The photosynthetic ability of green tissues associated with the presence of
chlorophyll was thought to improve resistance to freezing. However, somewhat contradictory results have been reported, there being no evidence of improved hardiness in cauliflower cultivars with green curds compared to those with white curds (Fuller et al. 1994).

(b) Environmental Factors

(i) Temperature

Plants can be hardened by exposing them for a few weeks to temperatures a few degrees above the freezing point. Maximum hardening of greenhouse grown cabbage seedlings at 3 °C resulted in a freeze-killing point of –7 to –10 °C. However, cabbage seedlings grown in growth chambers (25/15 °C day/night) and hardened for 6 weeks at successively lower temperatures from 5 °C to –3 °C attained a freeze-killing point of –20 °C (Kohn and Levitt 1965).

(ii) Light

No hardening of cabbage seedlings occurred in the dark at 4 °C or in the light at 18 °C, but hardening occurred when they were exposed to both low temperature and light. In cabbage, 8 J s⁻¹ m⁻² was sufficient for hardening to a tolerance of –7 °C, and some hardening was obtained at 5 J s⁻¹ m⁻² (Levitt 1980). Much greater hardening of cabbage (i.e. survival at -20 °C) was obtained with illumination at 43 J s⁻¹ m⁻² and cooling through a series of successively lower hardening temperatures (Kohn and Levitt 1965).

(iii) Photoperiod

In many herbaceous plants, hardening is improved by short photoperiods. However, in cabbage, photoperiod did not have any effect on cabbage growth or cold hardiness. Any effect of photoperiod on cabbage growth was due to the effect of total net photosynthate accumulated per day (Kohn and Levitt 1965).
Different degrees of freezing tolerance are inherent in different plant organs. In cabbage, the ranking in the order of increasing freezing tolerance was petiole < upper pith (stem) < middle pith < lamina (leaf) < lower pith. Inner and outer petiole tissues were killed at –8 °C. All stem pith and leaf lamina tissues survived –8 °C without injury. At –12 °C, only the lower pith survived without injury and, at –16 °C, all tissues were killed (Manley and Hummel 1996).

It has been suggested that the hardiness mechanism in foliar portions of the cauliflower plant is not expressed in the curd. Since cauliflower curd is incapable of surviving freezing, the only method of frost tolerance is by freeze avoidance via supercooling. Initial work by exotherm detection showed a range of freezing points from –1 to –6.3 °C (Grout et al. 1982). Further work by other researchers showed curd material, when frozen as isolated florets, supercooled over the range of –1 to –12 °C. The mean freezing point of the florets was -6.4 °C (Fuller et al. 1994). Curd florets which supercooled but did not freeze were completely undamaged, whereas freezing always led to cell damage and death. The large range of freezing points measured suggested a range of active ice nucleators either on or within the florets. When curds were frozen intact, the ability of florets to supercool was severely restricted. This was attributed to the seeding of freezing by the internal growth of ice crystals via interconnecting vascular bundles (Fuller et al. 1994).

(d) Stage of development

There is limited information on the stage of broccoli development that is most sensitive to sub-zero temperatures.

(i) Vegetative stage

The vegetative stage is thought to be the most tolerant stage as no frost sensitive reproductive parts have been initiated during this stage. Radiation frost treatments
between −2 to −4 °C for 0-4 days did not induce apical abortion (‘blindness’) in broccoli cultivars, ‘Arcadia’, ‘Marathon’, ‘Shogun’ and ‘Packman’ (Forsyth et al. 1999). Freezing tests on brassica oilseed seedlings at the 5-leaf stage determined killing temperature for 50% of the population (LT50) at −14 °C (Andrews and Morrison 1992).

(ii) Floral initiation stage

During FI, apical meristems should be able to avoid freezing to a certain extent due to the thermal insulation provided by the wrapper-leaves (Tapsell et al. 1990). The sensitivity of broccoli to sub-zero temperatures at FI is not known.

(iii) Inflorescence development stage

Initially, broccoli inflorescences may be insulated by wrapper-leaves. However, as the inflorescence enlarges, the wrapper-leaves slowly unfold, leaving the broccoli inflorescences exposed to the environment, including sub-zero temperatures (Tapsell et al. 1990). For the cauliflower curd, the only method of frost resistance is by freeze avoidance via supercooling (Fuller et al. 1994). The ability of broccoli inflorescence to supercool is not known.

2.6 Effect of other environmental factors

2.6.1 Effect of photoperiod

Many researchers working on temperature effects on broccoli have assumed that photoperiod does not have a modifying effect on the FI or flowering (Miller et al. 1985, Miller 1988, Marshall and Thompson 1987a, 1987b). Photoperiod had a negligible effect (accounting for only 1.2% of error variance) on rate of development
(Titley 1985). No evidence for photoperiod sensitivity was found in three commercial cauliflower cultivars (‘Plana’, ‘Kathmandu Local’ and ‘Snowball-16’) growing under different photoperiods (9, 12, 15, 18 h day\(^{-1}\)) in the UK (Thapa 1994, Hadley and Pearson 1998).

A highly significant interaction between temperature and photoperiod (irradiance of 86-95 J s\(^{-1}\) m\(^{-2}\), equivalent to 3.7-4.1 MJ m\(^{-2}\) day\(^{-1}\) for a 12 h day) reported in broccoli cultivar ‘Coastal’ for both chronological and developmental time to buttoning (Gauss and Taylor 1969b) was probably due to the higher total radiation received and not photoperiod per se. Plants grown at 13 °C showed a marked reduction in the time to buttoning as photoperiod was increased from 8 to 24 h. Time to buttoning for plants grown at 29 °C also decreased as photoperiod was increased from 8 to 16 h, but time to buttoning was increased under a 24 h photoperiod (Gauss and Taylor 1969b). Total leaf number was increased from 24 to 32 leaves at 29 °C but decreased from 17 to 13 leaves at 13 °C, as photoperiod was increased from 16 to 24 h. The authors hypothesised that the combination of high temperature and continuous light promoted rapid vegetative growth and retarded reproductive development (Gauss and Taylor 1969b). They thought that continuous light possibly converted P\(_{r}\) (phytochrome red) to P\(_{fr}\) (phytochrome far-red), and low temperature, simultaneously retarded breakdown or reversion of P\(_{r}\) to P\(_{fr}\) resulting in both chronological and developmental earliness of FI and buttoning. Reversion of P\(_{fr}\) to P\(_{r}\) can be retarded by low temperature in cauliflower and other plants (Hillman 1967). Presence of P\(_{fr}\) was thought to promote early flowering in some long-day plants (at the end of the dark period). Broccoli was classified as a quantitative long-day plant (Friend 1985), and thus would be expected to respond to long photoperiods (e.g. 16 h) by earlier FI.

Two very early cultivars (‘Gokuwase-midori’ and ‘Dark Horse’), three early cultivars (‘Wase-midori’, ‘Shaster’ and ‘Ryoku-yo’), and an intermediate cultivar (‘Three Seven’), reached FI one week later and had higher leaf number under short-day (8 h) than long-day (16 h) photoperiod at 17 °C (Fujime et al. 1988). Low temperature treatment was more effective than long-day treatment for photothermal induction of FI in broccoli plants.
2.6.2 Effect of solar radiation

To date, most researchers have worked on development and maturity models for crop scheduling based only on TT. Temperature was reported to explain 74.3% of the variation whereas solar radiation a further 17.7% of the variation, in a model developed to predict maturity in broccoli (Marshall and Thompson 1987a). Further work on broccoli maturity prediction models (Wurr et al. 1991a, 1992, Mourao and Hadley 1998) also incorporated a solar radiation component. In contrast, other authors have reported that there was no relationship between broccoli development and solar radiation (Fujime and Okuda 1994, Pearson and Hadley 1988). Broccoli plants grown under shade covers with solar radiation transmission of 62–75% were reported to develop a compensating mechanism such that leaf area ratio and specific leaf area increased to increase light interception (Mourao and Hadley 1998). The rate of conversion of this intercepted radiation with dry matter also increased. Solar radiation had no apparent effect on time to FI, but head growth after FI was influenced by solar radiation (Mourao and Hadley 1998).

The predictors of crop development, solar radiation and temperature may not be independent. There was a strong curvilinear relationship between solar radiation and temperature in the Scottish prediction model (Marshall and Thompson 1987a). This was due to the two predictor variables having the same periodic variation in time (an annual cycle). Since the predictors are not independent, inclusion of solar radiation into thermal time models may not improve the models. Solar radiation was not included in the TT model for predicting harvest maturity developed in Aarslev, Denmark, since inclusion of solar radiation did not improve the accuracy of the model for practical purposes (Grevsen 1998).

2.7 Models for predicting development and maturity

Crop scheduling can be defined as ‘a conscious directing of different steps in the production process combining all the different factors in the most economic way to ascertain the time of harvest as accurately as possible’ (Jensen 1980). A well-planned
production program is essential to maintain continuity of supply of broccoli to markets. Long term cold storage of broccoli to even out supply has limited application, due to the highly perishable nature of fresh market broccoli (Klieber and Wills 1991).

Staggered sowing dates of cultivars with contrasting maturity can be used to produce a planned production sequence (Pearson and Hadley 1988), but such a plan cannot allow, for example, for abnormal weather conditions, such as the very cool winters of 1994 and 1995 on the Darling Downs area of south-east Queensland (Jauncey, P. 1996, pers. comm.). Most of the research on crop scheduling of broccoli involved cultivar evaluations, in time of sowing experiments. The choice of cultivar is very important. By selecting cultivars of differing maturity, it is possible to extend the broccoli growing season. Time of sowing is also very important, as environmental factors such as temperature, photoperiod and solar radiation vary throughout the year, and may affect the rate of growth and development. There are essentially two temporal variations in climatic conditions: (i) the seasonal pattern that occurs on a regular cycle (e.g. photoperiod) and (ii) short term variations due to prevailing weather conditions (e.g. temperature). Solar radiation is also reduced by cloud cover.

Prediction of phenological events is crucial in crop scheduling as there may be different cardinal temperatures for different phenological intervals (Arnold 1959, Wang 1960, Diputado and Nichols 1989). Accurate prediction of phenological events will also assist farmers to plan fertiliser, irrigation and pesticide application programs which may be adjusted according to phenological stage.

A summary of models for prediction of phenological events and maturity of broccoli is presented in Table 2.8. Most of the models (Table 2.8) were developed in the temperate zones, and the Gatton models have not been used since they were difficult to interpret and apply. The strengths and weaknesses of individual models mentioned in Table 2.8 are discussed below:

2.7.1 Forthside model
The Forthside model was developed in Tasmania for predicting maturity time for a once-over harvest of broccoli using chronological time (days) from sowing to harvest maturity (Chung 1981). An alternative simple TT model was also mentioned but not recommended for use in Tasmania. The computation of simple TT suggested in the Forthside model assumes that plant growth is directly related to the average daily temperature above a $T_{\text{base}}$ (Arnold 1959) and the equation was defined as:

$$\text{Daily degree-day} = \left(\frac{T_{\text{max}} + T_{\text{min}}}{2} - T_{\text{base}}\right)$$  \hspace{1cm} (2.2)

where $T_{\text{max}}$ and $T_{\text{min}}$ are daily maximum and minimum temperatures respectively during the growing season and $T_{\text{base}}$ is a base temperature.
Table 2.8. Models for the prediction of phenological development and maturity of broccoli.

<table>
<thead>
<tr>
<th>Model</th>
<th>Location</th>
<th>Phenological interval</th>
<th>Brief description of model</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forthside model</td>
<td>Tasmania, Australia</td>
<td>Sowing to HM</td>
<td>Simple linear model predicting maturity based on chronological time (days) from sowing to harvest maturity.</td>
<td>Chung (1981)</td>
</tr>
</tbody>
</table>
| Gatton          models        | Gatton, Queensland, Australia | Sowing to HM | Three models to predict time to maturity were developed.  
  a) Non-linear, rectangular hyperbola model using mean temperature as the predictor for days to maturity (Mead et al. 1993).  
| Scottish model              | Scotland, UK      | Sowing to HM          | Model which assumes that crop progresses towards maturity when air temperature is above Tbase. Based on a multiple linear regression with thermal time and solar radiation as predictors. | Marshall and Thompson (1987a, 1987b) |
| Massey model                | Palmerston North, New Zealand | Sowing to FI to HM | Thermal time model based on observations of phenological stages.  
  Crop development divided into two stages, sowing to FI and FI to HM. A different Tbase was calculated for each stage. | Diputado and Nichols (1989) |
| Reading model               | Reading Univ., UK | FI to HM              | Prediction based on linear regression between natural logarithm of curd diameter and accumulated thermal time from FI to HM. | Pearson and Hadley (1988)   |
| Kagawa model                | Kagawa, Japan     | Sowing to buttoning, Sowing to HM | Models predicting days to buttoning and HM based on multiple regression analysis. The predictors used were maximum, minimum and integrated mean air temperatures. | Fujime and Okuda (1994)    |
| Wellesbourne vernalisation model | Wellesbourne, UK | Transplant. to FI | Model predicting the increase in apex diameter of broccoli with temperature. | Wurr et al. (1995)         |
| Wellesbourne maturity prediction models | Wellesbourne, UK | FI to HM              | Quadratic and logistic regressions between natural logarithm of head diameter and effective thermal time (ETT). The predictor, ETT is a function of thermal time and solar radiation (Scaife et al. 1987). The model was further developed to take plant density into account. | Wurr et al. (1991a, 1991b, 1992), Wurr 1992 |
| Clemson          model       | Clemson Univ., USA | Sowing to HM          | Prediction of harvest dates from sowing using thermal time. | Dufault (1997)              |
| Aarslev model            | Aarslev, Denmark  | FI to HM              | Quadratic regressions between natural logarithm of head diameter and thermal time. | Grevsen (1998)              |

The appropriate $T_{base}$ was calculated as the temperature which produces the lowest coefficient of variation in TT (Arnold 1959). $T_{base}$ for the cultivars ‘Futura’, ‘Gem’
and ‘Remco’ were found to be -4, -1, and -2 °C and thermal time from sowing to harvest maturity were 1830, 1537 and 1686 °C d above $T_{base}$, respectively (Chung 1981).

Although thermal time methods were suggested, the Forthside model developed was based on chronological time (days) from sowing to maturity and not temperature. Forthside weather records for twelve years, 1968-79 were analysed to give the following general relationships between sowing date and optimum date for harvest:

- ‘Futura’ - $y = 1.60x - 5.29$
- ‘Gem’ - $y = 1.79x - 0.20$
- ‘Remco’ - $y = 1.65x - 4.87$

where $x$ is the sowing time in days after 20 January, and $y$ is the predicted maturity time, in days after 21 April for ‘Gem’ or 26 April for ‘Futura’ and ‘Remco’. The Forthside model was developed specifically for the three cultivars to be grown in locations close to Forthside, Tasmania. Application of this model is site specific, and it may not be applicable to other locations with different climatic conditions.

2.7.2 Gatton models

The Gatton models were developed at Gatton College, south-east Queensland (Titley 1981, 1985, 1987, Fyffe and Titley 1989). Fifteen cultivars representing a range of maturities were evaluated in a time of sowing study (Titley 1981). Crop scheduling was based on the prediction of the duration from sowing to HM, using the three proposed models (Models “a”, “b” and “c”) described as follows:

(a) Non-linear, rectangular hyperbola model

Days from sowing to HM were calculated using the non-linear, rectangular hyperbola model (Model “a”), $t/d = a + bt$ (Mead et al. 1993) which was rearranged as follows:

$$d = t / (a + bt)$$

(2.3)
where \( t \) = mean temperature (°C), \( d \) = days from sowing to HM, and \( a \) & \( b \) are fitted constants.

(b) Simple thermal time model

The second model evaluated was the TT model (Model “b”) (Arnold 1959, Angus et al. 1981a) which was similar to (equation 2.2) used by Chung (1981). However, a fixed \( T_{base} \) of 4.5 °C was used for TT calculations for all cultivars.

(c) Modified thermal time (Barger System) model

The Barger system uses a base temperature-cutoff temperature (\( T_{base} - T_{opt} \) ‘heat stress’ modified TT formula (Model “c”). Using this approach, all recorded temperatures below \( T_{base} \) are considered equal to \( T_{base} \). All such temperatures above \( T_{opt} \) are considered equal to \( T_{opt} \). These values are substituted in Equation 2.2 (Arnold 1974). The modified TT were calculated for set \( T_{opt} \) of 30 °C, 27 °C, 24 °C, 21 °C and 18 °C. For all modified thermal time calculations, \( T_{base} \) was set at 4.5 °C (Fyffe and Titley 1989). The minimum standard deviation and lowest coefficient of variance was found when modified TT were obtained using \( T_{base} \) and \( T_{opt} \) of 0 °C and 21 °C, respectively, for all cultivars (Titley 1985).

(d) Comparison of Gatton models

The models developed originally on 1979-80 sowing experiments were tested using independent data from 1983-84 sowings. The relationship between observed days (from 1983-84 sowings) and predicted days (calculated based on parameters from 1979-80 sowings) to HM was analysed by linear regression in the form: \( y = a + bx \), where \( y \) is the observed duration in days, \( x \) is the predicted duration in days, \( a \) is the intercept, and \( b \) the regression coefficient. Simple (Model “b”) and modified (Model “c”) TT were also analysed in a similar equation replacing days with TT. The use of Model “a” (non-linear rectangular hyperbola model) to predict days for scheduling cultivars accounted for 85% of the variation compared to 23% and 64% using Models b and c, respectively (Titley 1985, 1987). Similar results, demonstrating that
estimates of days from mean temperatures for duration of sowing to HM were more accurate than simple or modified TT, were reported in subsequent work (Fyffe and Titley 1989). TT calculated using a fixed $T_{\text{base}}$ of 5 °C did not explain much of the variation in duration from sowing to harvest maturity for 14 hybrid cultivars grown under different environmental conditions (Hulbert and Orton 1984).

A possible explanation for lack of precision in TT techniques is that only one fixed $T_{\text{base}}$ was used for all cultivars and across all phenological stages, ignoring the value of 0 °C for $T_{\text{base}}$ derived by Titley (1985, 1987). For example, the Gatton models set a $T_{\text{base}}$ of 4.5 °C (Titley 1985, 1987, Fyffe and Titley 1989) while Hulbert and Orton (1984) set a $T_{\text{base}}$ of 5 °C. The nature of the error introduced into a linear TT system is that when the selected $T_{\text{base}}$ is too high, TT required for a particular cultivar will increase as mean temperature during the developmental period increases, and if the selected $T_{\text{base}}$ is too low, the reverse trend will take place (Arnold 1959). Hence, when $T_{\text{base}}$ is too high, chronological time for sowing to FI and HM is over-predicted at a cooler site and under-predicted at a warmer site, and if $T_{\text{base}}$ is too low, the reverse occurs. Critiques of the TT approach (Wang 1960) also pointed out that cardinal temperatures used for calculating TT should be changed according to phenological stage. Work in New Zealand (Diputado and Nichols 1989) has also shown that a different $T_{\text{base}}$ is required for each phenological stage in broccoli. A different $T_{\text{base}}$ for different phenological stages has also been observed in other crops such as maize ($Zea mays$ L) (Birch 1996). Greater precision in TT calculations may be possible by selecting the correct $T_{\text{base}}$ for each cultivar.

### 2.7.3 Scottish model

The Scottish model was developed at the Scottish Crop Research Institute, for predicting maturity (Marshall and Thompson 1987a, 1987b). The model assumes that the crop progresses towards maturity when air temperature is above $T_{\text{base}}$. This model is based on a multiple linear regression using the two predictors, TT accumulated above $T_{\text{base}}$, and solar radiation, to predict duration from sowing to HM. TT accounted for 74.3% of the variation and solar radiation a further 17.7%. Given daily climatic records, maturity could be predicted within ±7 days for nine out of ten crops.
over the four years considered. Within any one year, the precision improved to ±5 days. Accuracy of prediction was poor since a single model was used for the whole crop duration and the predictors, temperature and solar radiation were not independent. The authors suggested that any major advances in the future are likely to come from models based on observations of phenological stages of the crop (Marshall and Thompson 1987b). This suggestion was also supported by later work (Wang 1960, Diputado and Nichols 1989, Wurr et al. 1991a, 1992, Wurr 1992).

2.7.4 Massey model

The Massey model was developed at Massey University in Palmerston North, New Zealand (Diputado and Nichols 1989). TT above various $T_{\text{base}}$ was calculated for each sowing date based upon the following formulae:

\[
\begin{align*}
\text{if } T_{\text{min}} > T_{\text{base}}, & \quad TT = T_{\text{mean}} - T_{\text{base}} \quad (2.4) \\
\text{if } T_{\text{min}} < T_{\text{base}} \text{ and } T_{\text{mean}} > T_{\text{base}}, & \quad TT = (T_{\text{max}} - T_{\text{base}})/2 - (T_{\text{base}} - T_{\text{min}})/4 \\
\text{if } T_{\text{mean}} < T_{\text{base}} \text{ and } T_{\text{max}} > T_{\text{base}}, & \quad TT = (T_{\text{max}} - T_{\text{base}})/4 \\
\text{if } T_{\text{max}} < T_{\text{base}}, & \quad TT = 0.0
\end{align*}
\]

where: $TT = \text{Thermal time}; T_{\text{max}} = \text{maximum temperature}; T_{\text{min}} = \text{minimum temperature}; T_{\text{mean}} = (T_{\text{max}} - T_{\text{min}})/2; T_{\text{base}} = \text{base temperature}.$

The above formulae will be similar to (equation 2.2) when $T_{\text{base}} = 0 \degree \text{C}$ for cultivars growing in a warm sub-tropical environment (eg. Gatton) where $T_{\text{min}} > 0 \degree \text{C}$ during the growing season in an average year. Total duration from sowing to maturity was divided into two phenological stages:

a) Sowing to FI; and
b) FI to HM

The $T_{\text{base}}$ and $T_{\text{max}}$ yielding the least coefficient of variation were considered to be most appropriate and were calculated as follows:

<table>
<thead>
<tr>
<th>Phenological interval</th>
<th>$T_{\text{base}}$</th>
<th>$T_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Sowing to FI</td>
<td>1 $\degree \text{C}$</td>
<td>21 $\degree \text{C}$</td>
</tr>
<tr>
<td>b) FI to HM</td>
<td>3 $\degree \text{C}$</td>
<td>-</td>
</tr>
</tbody>
</table>
The relationship between the natural logarithm of head diameter and TT (calculated using the appropriate $T_{\text{base}}$) was then fitted into a simple linear model. There appeared to be a wider variation in TT for the first stage (sowing to FI) compared with the second stage (FI to HM). This same phenomenon was also observed by Wurr et al. (1991a) who thought that sowing to FI represented satisfaction of the crops’ vernalisation requirement, while FI to HM was the response of head growth to the environment.

By using the appropriate $T_{\text{base}}$ to calculate TT for different phenological stages (Diputado and Nichols 1989), precision of the TT technique was improved as TT can predict time to HM more accurately than the set $T_{\text{base}}$ of 4.5 °C used in the Gatton models (Titley 1985, 1987). However, variation in the time from sowing to HM may not be accounted for by temperature alone, and influence of other environmental influences such as photoperiod, should not be ignored.

2.7.5 Reading model

The Reading model was developed at University of Reading, UK to predict head growth under plastic film crop covers (Pearson and Hadley 1988). This model is very similar to the Massey model except that it only covers one phenological stage, FI to HM. The natural logarithm of head diameter was found to increase linearly with accumulated TT from FI to HM. However, $T_{\text{base}}$ was found to be different for different cultivars. $T_{\text{base}}$ for cultivars ‘Mecedes’ and ‘Corvet’ was reported to be 7 °C and 0.6 °C respectively. The authors also mentioned that they did not find any evidence that solar radiation affects curd growth in any significant way, but they did not present data to support this.

2.7.6 Kagawa model

The Kagawa model was developed at Kagawa University, Japan, for predicting buttoning and harvest time of transplanted broccoli grown under field conditions (Fujime and Okuda 1994). A different approach to the TT technique was used. Multiple regression analysis were used, with temperature data at different intervals as
predictors, rather than TT. Seedlings of cultivar ‘Wase-midori’ were transplanted at the 6-7 leaf stage in spring, and environmental data including mean, maximum, minimum and integrated mean air temperatures, mean and integrated solar radiation and mean and integrated soil temperatures (50 mm below soil level) were recorded daily. The average and integrated mean values of all factors were calculated for 10-day intervals from transplanting.

High and negative relationships were found between the days from transplanting to HM and the minimum air temperature and integrated mean air temperature during the transplanting times, as well as the maximum air temperatures and integrated mean air temperatures from 10 to 50 days after transplanting as shown below:

\[ y = 214.932 + 4.297x_1 - 7.706x_2 - 3.645x_3 - 6.919x_4 + 0.293x_5 \] (2.5)

where  
- \( y \) = days from transplanting to HM
- \( x_1 \) = minimum air temperature during the transplanting period
- \( x_2 \) = maximum air temperature at 10 days after transplanting
- \( x_3 \) = maximum air temperature at 20 days after transplanting
- \( x_4 \) = maximum air temperature at 30 days after transplanting
- \( x_5 \) = integrated mean air temperature until 40 days after transplanting

The authors commented that there was almost no relationship between days from transplanting to HM, and solar radiation. It was observed that the higher the minimum air temperature at sowing and, thereafter, the higher the air and soil temperature, the earlier the harvest time. This model is probably only stable within a limited temperature range. For example, at a constant temperature of 0 °C, \( y = 215 \) days. This is not physiologically possible, because 0 °C was reported to be \( T_{\text{base}} \) for broccoli development by several researchers (Marshall and Thompson 1987a, Wurr et al. 1991, Grevsen 1998), and development should not proceed at and below 0 °C.

The predictors in the multiple regression may also not be independent. The coefficients have three decimal places and such precision is rather unconvincing. The Kagawa models are empirical relationships with little physiological base and may not be applicable to other environments.
2.7.7 Wellesbourne vernalisation model

The Wellesbourne vernalisation model for broccoli was developed in Wellesbourne, UK to predict change in apex diameter with temperature from transplanting to FI (Wurr et al. 1995). This model required numerous microscopic examinations of shoot apex and controlled environment conditions for its development. A major limitation for vernalisation models is the lack of quantitative models for predicting the cessation of juvenility in broccoli, beyond the fact that plants require the production of a similar number of leaves (4 leaves > 2 cm) (Wiebe 1990) before they become receptive to inductive temperatures. Although this vernalisation model did not include a juvenile phase, transplanted seedlings were at the 8-leaf stage at the time of transplanting, and could have passed the juvenile phase before transplanting. The only indication that there might be a juvenile phase, is the presence of parameter ‘a’ - the diameter value after which subsequent increase is exponential. Nevertheless, the plant has to grow for several weeks and produce a minimum stem diameter and number of leaves before being receptive to vernalising temperatures. This implies that TT techniques may still be useful for prediction of FI during developmental stage 2 (Pearson et al. 1994, Hadley and Pearson 1998).

2.7.8 Wellesbourne maturity prediction models

The Wellesbourne maturity prediction models for broccoli were developed at Wellesbourne, UK (Wurr et al. (1991a, 1991b, 1992). The two models developed are briefly described as follows:

(a) Quadratic transplanting to HM model

A model fitting parallel quadratic curves of cultivars ‘Citation’, ‘Prima’ and ‘Cruiser’ was developed as follows:
\[ d_{TM} = a + bd_T + cd_T^2 \]  

(2.6)

where \( d_{TM} \) = days from transplanting to HM, \( d_T \) = day of transplanting. The curves for different cultivars differ in the ‘a’ parameter only, and ‘b’ and ‘c’ are fitted coefficients. This model accounted for 76\% of the variance in time from transplanting to HM and could be used to plan continuity schedules in Wellesbourne.

\((b)\) Maturity prediction from FI to HM

An improvement on the Massey and Reading models for predicting growth of head diameter from FI and HM was made by use of solar radiation measurements as well as TT as predictors. Environmental variables accounting for head growth of five cultivars were studied (Wurr et al. 1991a), and accumulated effective thermal time (ETT) (Scaife et al. 1987) accounted for more variation (in head growth from FI to HM) than either time, solar radiation, or TT. The ETT for each day were calculated as:

\[ \frac{1}{ETT} = \frac{1}{TT} + \frac{a}{R} \]  

(2.7)

where TT = thermal time for the day, \( R \) = total radiation in MJ m\(^{-2}\) for the day, and \( a \) = a unitless constant. A logistic relationship between the natural logarithm of head diameter and accumulated ETT, gave the best fit (accounted for 96\% of variation) but differences between quadratic, logistic and Gompertz curves were trivial, and all three curves could be used to describe head growth adequately.

Since a logistic model requires estimates of four parameters, a quadratic model:

\[ y = ax^2 + bx + c \]  

(2.8)

where \( y \) = natural logarithm of head diameter, \( x = ETT \), and ‘a’, ‘b’, and ‘c’ are independent fitted parameters, requires only three parameters and was considered to be simpler (Wurr et al. 1992). With the cultivar ‘Cruiser’, ETT with a \( T_{base} \) of 0 °C and an upper limit of 13 °C was most appropriate. The derivation of both ETT and

To adjust equation 2.8 for a crop grown at $\rho$ plants m$^{-2}$, the relationship may be modified as follows:

$$y = ax^2 + b_\rho x + \ln (0.49)$$  \hspace{1cm} (2.9)

where $y$ = natural logarithm of head diameter, $x$ = ETT from FI to HM, $b_\rho = \lambda_\rho + z$ (Wurr et al. 1992), $\ln (0.49)$ = a constant at FI (Wurr et al. 1995), $\rho$ = plant density (plants m$^{-2}$), and ‘a’, $\lambda$ and ‘z’ are independent fitted parameters.

The accuracy of predictions made by the model was tested on independent samples of the cultivar ‘Cruiser’ grown at densities from 5 to 15 plants m$^{-2}$. For crops grown at 5 plants m$^{-2}$, the target head diameter was 110 mm, and for crops grown at 15 plants m$^{-2}$, the target head diameter was 75 mm. Using the observed head diameters at maturity, and observed meteorological data, the test resulted in a mean deviation of -0.6 days and a prediction error (RMSD) (Mikkelsen 1981) of 1.73 days. Using average meteorological data and the target diameters resulted in a mean deviation of -1.3 days and a prediction error of 3.26 days (Wurr 1992). Predictions were very accurate using actual head diameters and observed weather but were less accurate when using target diameters and average weather (Wurr et al. 1992, Wurr 1992).

Further testing of the model was performed on commercial broccoli crops in Fife, Scotland and Lincs. The accuracy of maturity predictions was good with cultivars ‘Shogun’, ‘Marathon’, ‘Skiff’ and ‘Cruiser’ but the use of the models for predictions of maturity with cultivars ‘Caravel’, ‘Arcadia’ and ‘Greenbelt’ was not recommended (Wurr et al. 1991b). This implies that the maturity prediction model works better on some cultivars than others. A computer program, Broccoli®, has been developed in the UK which aids prediction of harvest dates. This computer program predicts maturity dates for six broccoli cultivars using meteorological and crop data (Horticultural Development Council 1994, Wurr 1995).
The Wellesbourne models appear to be the most accurate and well tested models in the literature. However, these models require frequent measurements of head diameter, solar radiation and knowledge of cultivar specific sensitivity to solar radiation which has to be derived from years of experimentation. Application of ETT as a predictor may be useful in a temperate environment, but inclusion of solar radiation may not improve thermal time models in a warmer sub-tropical environment at lower latitudes where temperatures are close to optimum and solar radiation may not influence development. In these environments, there is usually enough radiation to saturate photosynthetic requirements (Birch et al. 1998b)

2.7.9 Clemson model

In the Clemson model developed at Clemson University, USA (Dufault 1997), the least variable method using coefficients of variation was used to predict harvest dates based on thermal time summation. The method with the lowest coefficient of variation for predicting first harvest was to sum, over days from sowing to harvest, the difference between the mean temperature \([T_{\text{max}} + T_{\text{min}}]/2\) and a \(T_{\text{base}}\) of 7.2 °C, similar to the equation in the Forths ide Model (Equation 2.2) (Arnold 1959, Chung 1981) when \(T_{\text{max}} \leq 26.7 \degree \text{C}\). When \(T_{\text{max}} > 26.7 \degree \text{C}\), \(T_{\text{max}} = [(T_{\text{max}} - C) - (T_{\text{max}} + T_{\text{min}})]/2\) where \(T_{\text{max}} = \) maximum temperature, \(T_{\text{min}} = \) minimum temperature, \(C = \) ceiling temperature (26.7 °C). The calculation for \(T_{\text{max}}\) does not seem to be mathematically correct when \(T_{\text{max}} > 26.7 \degree \text{C}\). When actual temperatures are substituted into the equation, negative figures are obtained, which have no biological basis. Moreover, the thermal time calculations do not account for any effect of solar radiation or photoperiod sensitivity which may affect broccoli development.

2.7.10 Aarslev model

The Aarslev model was developed in Aarslev, Denmark to predict head growth using thermal time (Grevsen 1998). This model uses equations modified from Equations 2.8 and 2.9 in the Wellesbourne models (see Section 2.7.8). However, this predictive model was based on thermal time only, with effects of density and cultivar included. Optimised values of \(T_{\text{base}}\) and \(T_{\text{opt}}\) were derived by minimising the residual sum of
squares in the quadratic relationship between natural logarithm of head diameter and thermal time. Thermal time was calculated from optimised $T_{\text{base}}$ and $T_{\text{opt}}$ of 0 and 17 °C, respectively. Solar radiation was not included in this model since inclusion of solar radiation only gave a minor improvement in precision. The Aaslev model requires frequent measurements of head diameter similar to the Wellesbourne maturity model, which may not be practical for use by farmers.

### 2.7.11 Evaluation of prediction models

Some thermal models use the same $T_{\text{base}}$ from sowing to harvest (e.g. Forthside, Gatton, Scottish, Clemson and Grevsen models) while others use slightly different $T_{\text{base}}$ according to phenological stage (e.g. Massey model). Specific models have also been developed to predict floral initiation (e.g. Wellesbourne vernalisation model) and harvest maturity from floral initiation (e.g. Reading, Wellesbourne and Aarslev maturity models). There has been no attempt to compare the models or compare the advantage of using different models for different developmental stages with a single model for the whole crop duration. However, such comparisons will be of limited value, since many of the models suffer from either site specificity or are not based on physiological processes as outlined in the following paragraphs.

Site specific models such as the Forthside model are not generally useful, since they cannot be applied at other locations. Precision of the simple thermal time (equation 2.2) and modified (Barger system) thermal time Gatton models can be improved by using the appropriate $T_{\text{base}}$ of 0 °C rather than a set $T_{\text{base}}$ of 4.5 °C derived for sweet corn (*Zea mays* L). The use of a non-linear, rectangular hyperbola (equation 2.3) model, based on mean temperatures in Gatton, is similar to a simple thermal time model with $T_{\text{base}}$ of 0 °C, but has a disadvantage in that it requires two cultivar-specific constants to be derived, and these constants may be location specific and difficult to interpret.

The Massey, Clemson, Reading and Gatton models are based on temperature responses only and other environmental effects such as photoperiod and solar radiation have not been evaluated. Application of a single model for the whole crop
duration was the likely cause for lack of accuracy in the Scottish model. Also predictors were not independent. The Kagawa models are empirical relationships with little physiological base. The Aarslev model, and Wellesbourne vernalisation and maturity models are precise and well tested, but are complex and may not be practical or simple enough for application by farmers.

Hence, there is a need to develop a robust thermal time model for predicting broccoli development that is simple, precise and can be applied to a range of cultivars in different locations. Other environmental factors such as photoperiod and solar radiation should also be studied since these factors may interact with temperature and influence broccoli development.

### 2.8 Conclusions

The major gaps that emerge from this literature review are listed below. These need to be addressed in order to achieve the objectives stated in Chapter 1 (Section 1.5).

1. There is a discrepancy in the literature for describing broccoli floral initiation. A simple and precise field method for detecting floral initiation would be beneficial to farmers and researchers.

2. Little is known about the relative freeze sensitivity of broccoli at different developmental stages. Since there are no reports on the effects of sub-zero temperatures on yield and quality, research is needed for broccoli in growing areas such as the Darling Downs, where severe frosts are often experienced in winter.

3. Broccoli head quality is influenced by temperature. There is little research on temperature and photoperiod responses of yield and quality for broccoli grown in
a sub-tropical environment. Farmers can optimise yield and quality by matching cultivars to time of sowing through better understanding of genotype and environmental interactions.

4. Little is known about temperature and photoperiod responses of broccoli development in a sub-tropical environment, because broccoli development models were mostly developed in temperate latitudes. Many of these models suffer from site specificity, lack a physiological base, or are too complex.
Chapter 3
General Materials and Methods

Experiments were conducted to investigate the research questions and objectives identified in Chapters 1 and 2. To avoid repetition, this chapter describes the site and experimental methods that were common to the experiments in Chapters 4 to 8. Materials and methods specific to each chapter are described in the respective chapters.

3.1 Field experiment with photoperiod extension

A field experiment with photoperiod extension was conducted in 1997 at the University of Queensland, Gatton College (latitude 27°33’S, longitude 152°20’E, altitude 89 m), located in the Lockyer Valley, approximately 80 km west of Brisbane. Three broccoli cultivars, ‘Fiesta’ (Bejo Zaden BV, Holland), ‘Greenbelt’ and ‘Marathon’ (Sakata, Japan), were sown on eight dates (11 March, 20 March, 1 April, 10 April, 21 April, 01 May, 12 May, 22 May 1997), (sowings #1 to #8) under natural and extended (16 h) photoperiods. The natural photoperiods, including civil twilight (Jones and Kiniry 1986), were calculated for the period from when there were 4 leaves > 2 cm to harvest maturity, assuming the juvenile stage ended at this stage (Wiebe 1990). These photoperiods were 12.6, 12.4, 12.0, 11.8, 11.5, 11.4, 11.3 and 11.3 h for each sowing date, respectively, and they decreased by at most 30 min during the inductive phase of plants (Fig. 3.1) grown under natural photoperiod conditions. The soil type was a Black Earth (Blenheim Series) or Vertosol, typical of Lockyer Valley soils (Schafer et al. 1984, Isbell 1996).

A split split-plot experimental design with three replicates was used (Fig. 3.2), similar to that used in photoperiod extension studies for maize (Zea mays L.) (Birch et al. 1998a). Photoperiod treatment was the main plot, sowing date the sub-plot, and cultivar the sub-sub-plot, each randomised within the next higher level.
Fig. 3.1. Daily natural photoperiod (h) (curved line) at Gatton College from 1 February to 29 September. The day of the year is in Julian days (Jday) where 1 = 1 Jan. The horizontal line is the 16 h photoperiod treatment for the field experiment with photoperiod extension. S = sowing (1 to 8).

Three rows, 0.35 m apart and 8 m long were sown for each cultivar on raised beds for each sowing date in each sub-sub-plot. In all sowings, seeds were sown 0.1 m apart and seedlings thinned to 0.3 m plant spacing (6 plants m$^{-2}$) one week after emergence. There were 57 to 63 plants in each experimental unit (sub-sub-plot) guarded by 1 m of plants on each end. Forty plants (outer portion on each end) were used for destructive sampling for determination of floral initiation, while 20 plants (inner datum area) were used for non-destructive sampling for leaf number.

Photoperiod extension to 16 h was achieved by installing two rows of two lights (Philips RO 80 lights of output 100 W with reflective backs), the rows of lights being 3 m apart over the appropriate sub-plot (Plates 3.1a and 3.1b). The lights were maintained 1 m above the crop canopy. This arrangement produced a minimum of 2 W m$^{-2}$ at the canopy in the corners of the plots, measured with a LI-COR® Photometer, model LI-185A. The lights were automatically switched on at 0400 h and off at 0800 h, and on again at 1600 h and off at 2000 h daily. Photoperiod extension continued from sowing until harvest. Spill of light into neighbouring plots where the crop was grown under natural photoperiod conditions was prevented by the angle of downward projection of the light and a 3 m wide guard between the main plots.
Fig. 3.2. Field experiment layout (not to scale) used in a study of the effects of photoperiod extension, sowing date and cultivar on broccoli development, leaf number, yield and quality. S = Sowing (1 to 8), C = Cultivar (C1 = ‘Fiesta’, C2 = ‘Greenbelt’, and C3 = ‘Marathon’).
Plate 3.1. The arrangement used in the photoperiod extension experiment showing (a) method of illumination, and (b) general view of the experiment, with broccoli plants at various stages of development.

Irrigation and nutrients were supplied at rates to ensure that non-limiting conditions were maintained. A soil analysis showed that nitrogen, zinc and boron were deficient, when assessed against accepted standards (Anon. 1991). Nitrogen was applied at
100 kg N ha\(^{-1}\) 2 days before sowing, followed by 20 kg N ha\(^{-1}\) at 2, 4 and 6 weeks after sowing. Zinc was applied at 1 kg zinc sulphate heptahydrate (ZnSO\(_4\).7H\(_2\)O) in 100 L water ha\(^{-1}\), and boron was applied at 0.15 kg Solubor\(^{\circledR}\) (Na\(_2\)B\(_6\)O\(_{13}\).4H\(_2\)O) in 100 L water ha\(^{-1}\) as a foliar spray at 14-day intervals from emergence to harvest. Insect pests and weeds were controlled as required.

3.2 Commercial farm crops

Data were also obtained from a commercial farm, Matilda Fresh Foods Pty Ltd at ‘Wando’ (latitude 27°39’S, longitude 151°21’E, altitude 364 m), located near Brookstead (hereafter referred to as the Brookstead location) on the Darling Downs, approximately 200 km west of Brisbane, Queensland. Crops were sown in double rows 0.25 by 0.25 m (8 plants m\(^{-2}\)) on beds 1.0 m apart. The soil type was a fertile black, self-mulching cracking clay (Black Earth or Vertosol) typical of Darling Downs soils (Isbell 1996). Vigorous crop growth was assured by appropriate application of fertiliser, furrow irrigation and insecticides according to commercial practices.

3.3 Data collection

3.3.1 Climatic data

Daily maximum and minimum temperatures (°C) (Fig. 3.3a) and total solar radiation (MJ m\(^{-2}\), Kipp & Zonen\(^{\circledR}\) CM11 pyranometer) (Fig. 3.4a) were obtained from a standard weather station located approximately 100 m from the experimental site at Gatton College. Temperatures (Fig. 3.3b,c) and total solar radiation (Fig. 3.4b,c) for the commercial farm at the Brookstead location were obtained from an on-farm automatic weather station.
Fig. 3.3. Daily maximum and minimum temperatures (°C) at (a) Gatton College in 1997, (b) Brookstead in 1997 and (c) Brookstead in 1998 during the broccoli growing season from 1 February to 29 September. The day of the year is in Julian days (Jday) where 1 = 1 Jan. The horizontal lines are at 0 and 20 °C: the base and optimum temperatures for calculation of thermal time. S = sowing (1 to 8) for the field experiment with photoperiod extension at Gatton College (a).
Fig. 3.4. Daily solar radiation (MJ m$^{-2}$ day$^{-1}$) at (a) Gatton College in 1997, (b) Brookstead in 1997 and (c) Brookstead in 1998 during the broccoli growing season from 1 February to 29 September. The day of the year is in Julian days (Jday) where 1 = 1 Jan. S = sowing (1 to 8) for the field experiment with photoperiod extension at Gatton College (a).
3.3.2 Crop ontogeny

Time of emergence was recorded when 50% of the seedling hypocotyls had emerged from the soil.

Floral initiation was determined from three randomly selected plants, removed at 3-day intervals, from 40 plants in the outer portion of each sub-sub-plot, starting 35 days after emergence. The apices were dissected under a stereoscopic light microscope (at x100 magnification) and their morphological stage compared with standard electron micrographs (Tan et al. 1998, Chapter 4). The assessment continued until the apices rated 4 on a scale of 1 to 7. Floral initiation was recorded when the graph of apex rating against time from emergence reached 3 (Tan et al. 1997, 1998, Chapter 4).

The buttoning stage was defined as the stage at which the immature inflorescence (or button) was approximately 10 mm diameter (Gauss and Taylor 1969b, Marshall and Thompson 1987a).

All broccoli heads were harvested when 50% of each sub-sub-plot reached an inflorescence diameter of 100 mm (Dufault 1997, Grevsen 1998). This coincides with the developmental stage just before the buds start to open and is representative of the time of harvest to fill orders for the export grades for the Japanese and south-east Asian markets which require head sizes of 100-120 mm and 80-100 mm head diameter, respectively (Tan et al. 1997). Harvested heads were trimmed to a stalk length of approximately 120 mm and bracts longer than 10 mm were removed (Chung 1982).

3.3.3 Leaf number

The total number of leaves present was recorded at floral initiation. To ensure an accurate count of total leaf number, the tenth leaf on each plant was marked with adhesive plastic tape and used as a reference leaf.
3.3.4 Head quality

The following quality attribute assessments (scores in parenthesis) were made (Chapters 5 and 6) (Chowings 1974, Chung 1982, Dufault 1996, O’Donnell et al. 1996): head shape – deep (5) to shallow (1) (Plate 3.2a); branching angle – wide (5) to narrow (1) (Plate 3.2b); cluster separation – no obvious cluster (5) to bud clusters over the surface of head (1); evenness of bud size – all the same size (5) to less than 25% the same size (1); bud colour – dark green (5) to pale green (1); bud size – the largest bud of each head (mm); bractiness – number of bracts (protruding from the head) present; and, hollow stem – present or absent. Attribute ratings of 1 were least desirable (unmarketable) and ratings of 5 were most desirable (highly marketable). Attribute ratings of 1 and 2 were deemed unmarketable, while ratings of 3, 4 and 5 were deemed marketable.

3.4 Data analysis

Analysis of variance (ANOVA) was completed for data from the photoperiod extension field experiment (split split-plot design) at Gatton College to test the independent and interactive effects of photoperiod extension, sowing date and cultivar, using the general linear model (GLM) procedure of SAS version 6.12 (SAS Institute 1989).

Where there was a significant interaction between sowing date and cultivar, the l.s.d. of cultivar comparisons within and between sowing dates were calculated. The greater l.s.d. comparing cultivars between sowing dates was presented.

Regressions were calculated using SAS and plotted using the graphics package Sigmaplot (Kuo and Fox 1993).

Root mean square deviation (RMSD), equivalent to the prediction error (Mikkelsen 1981, Yan and Wallace 1998), was used to compare models.
Plate 3.2. Photographic representation of broccoli head showing (a) head shape [deep (5) and shallow (1)] and (b) branching angle [wide (5) and narrow (1)].
Aggregate data associated with the research reported in this thesis have been retained in the School of Land and Food (Gatton College) as required by The University of Queensland.

### 3.5 Summary

General methods used in the following chapters (Chapters 4 to 8) have been described in this chapter (Chapter 3) to avoid repetition. This chapter describes details of the experimental design and cultural practices for the photoperiod extension field experiment conducted at Gatton College, the location and soil type for commercial farm crops grown near Brookstead on the Darling Downs, and methods of data collection for climatic data, crop ontogeny, leaf number and head quality. Details of the statistical analysis used for the photoperiod extension experiment were also presented. The next five chapters will describe studies carried out to generate data to achieve the objectives outlined in Chapter 1.
Chapter 4

Detection of floral initiation based on electron micrograph standards of shoot apices

4.1 Introduction

Predictive models for broccoli maturity will be useful for farmers in planning production schedules to provide continuity of supply, and in timing cultural practices that are related to crop phenological stages, such as fertiliser application, irrigation, and crop protection (Theunissen and Sins 1984). Also, the detection of floral initiation is vital when developing crop models that predict ontogeny (Diputado and Nichols 1989).

Floral initiation is defined as the stage when a new reproductive structure first develops on a previously vegetative apex. Scanning electron micrographs (SEMs) provide clear, 3-dimensional images of the developmental stages of the shoot apex and field assessment of floral initiation can easily be done by dissecting a shoot apex under a light microscope and comparing it against electron micrographs. A set of scanning electron micrographs of the shoot apex is available for brassica oilseed (*B. napus* L.) (Polowick and Sawhney 1986) and cauliflower (Margara and David 1978, Moncur 1981), but not for broccoli. Moreover, broccoli is ontogenetically more mature than cauliflower at harvest maturity (see Fig. 2.1 in Chapter 2) (Wiebe 1975, Gray 1982, 1989).

Hitherto, histological methods have been used to study the morphological development of the apex of green sprouting broccoli (Gauss and Taylor 1969a) and cauliflower (Sadik 1962). The histological methods require tedious microscopic dissections and laboratory techniques, which are difficult and not appropriate for field assessment of floral initiation. Whilst several authors have reported the effect of environmental factors, such as temperature, on the timing of floral initiation in broccoli (Fontes *et al.* 1967, Gauss and Taylor 1969b, Wiebe 1975, Diputado and Nichols 1989, Fyffe and Titley 1989), most only gave brief descriptions of the apex
during transition from vegetative to reproductive tissue, and no diagnostic description of floral initiation. Furthermore, there are wide variations in the apex diameter when floral initiation was described, ranging from ≥ 490 µm (Wurr et al. 1995) to > 2000 µm (Fyffe and Titley 1989). These discrepancies are probably due to differences in interpretation of each developmental stage and to the different magnifications used to view the samples. Japanese researchers (Fujime and Hirose 1979, 1980, 1981, Fujime et al. 1988, Fujime and Okuda 1996) have used a developmental scale for the transition from vegetative to reproductive stages at the apex in broccoli and cauliflower, but their descriptions lack the required detail and illustrations.

The objective of this study was to provide a reliable and repeatable method of determining floral initiation in broccoli. This chapter presents a sequential series of scanning electron micrographs, supported by descriptions of the transition from the vegetative to reproductive apex. Floral initiation was identified in a field experiment at Gatton College by comparing broccoli apices under a light microscope against the electron micrographs. The technique proved to be repeatable and consistent across three cultivars in the field.

4.2 Materials and methods

A 2-step approach was taken.

The first step consisted of identifying floral initiation by describing the transition from a vegetative to reproductive apex using scanning electron micrographs of broccoli apices obtained from the Brookstead location.

The second step consisted of applying the description of the floral initiation stage to three cultivars growing in different environments at Gatton College.

4.2.1 Source of samples for scanning electron microscopy
Shoot apices of cultivar ‘Fiesta’ were obtained from the commercial farm at the Brookstead location described in Chapter 3 (Section 3.2).

4.2.2 Scanning electron microscopy

Twenty broccoli apices from Brookstead were randomly selected on 19, 23 and 29 August 1996, corresponding to 70, 74 and 80 days after sowing. The apices were removed and fixed in 3% glutaraldehyde solution, buffered to pH of 6.8 using 0.1 mol L\(^{-1}\) phosphate buffer, for 24 h. They were then washed in the buffer solution, transferred to osmium tetroxide for 12 h to improve conductivity, dehydrated in a graded series of acetone solution concentrations and dried in a critical point dryer. After drying, the apical meristems were mounted on stubs, sputter coated with gold, examined with a JEOL JSM 820 scanning electron microscope at an accelerating voltage of 10 kV, and the images of the five morphological stages photographed (see Plates 4.1b to 4.1f).

The diameter of the vegetative apex was the horizontal distance between the insertion of the two uppermost leaf primordia (Esau 1965, Gauss and Taylor 1969a) and was measured from the micrograph to the nearest 10 \(\mu m\). After floral initiation (see Section 4.3), the horizontal distance between the insertion of the outermost bract primordia of the reproductive apex was measured from the micrograph to the nearest 10 \(\mu m\) in two directions at 90° to each other (Wurr et al. 1991a, 1992). The mean of the two measurements was taken as the apex diameter (see Plate 4.1a).

4.2.3 Source of samples for light microscopy

Additional broccoli apices at floral initiation were obtained from the field experiment with photoperiod extension conducted at the University of Queensland, Gatton College. Details of the experimental design are described in Chapter 3 (Section 3.1). Briefly, three cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) were sown eight times under natural and extended (16 h) photoperiods.

4.2.4 Light microscopy
A technique was developed to detect floral initiation (Stage 3, see Section 4.3.1) by comparing shoot apices under a light microscope against the electron micrographs (Plate 4.1). Starting 35 days after emergence, triplicate apices of randomly located broccoli plants in each sub-sub-plot at Gatton College were removed every three days, sealed immediately in a polyethylene bag and cooled at approximately $4 \pm 1 \, ^\circ$C for 1 h. After cooling, the apices were dissected under an Olympus BH-2 stereoscopic light microscope ($\times 100$) and their morphological stage compared to the broccoli electron micrographs (Plate 4.1) produced from this study. The microscope was fitted with an eyepiece graticule calibrated against a standard stage micrometer. Reproductive apices at floral initiation (Stage 3, Plate 4.1d, Table 4.1) were selected and measured orthogonally.

**4.2.5 Statistical analysis**

The diameter of four apices for each of the five apex morphological stages, were recorded from the scanning electron micrographs, and subjected to a Kruskal-Wallis test using the nonparametric (NPAR1WAY) procedure of SAS (SAS Institute 1989). Analysis of variance was not carried out because the within-stage variance was not constant.

At Stage 3, mean apex diameter of the triplicate apices from each sub-sub-plot of the field experiment were subjected to a standard analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS (Chapter 3, Section 3.4).

**4.3 Results**

**4.3.1 Morphological development of the apical meristem**
The scanning electron micrographs for Stages 1 to 5 are shown in Plates 4.1b to 4.1f, respectively. The plate caption provides the method for measuring apex diameter (Plate 4.1a) and a brief description of Stages 1 to 5. A detailed description of the apex during the transition from vegetative to reproductive stages (morphological stages 1 to 7) is outlined in Table 4.1.
Plate 4.1. Morphological development of broccoli apical meristem. (a) Measurements used to calculate the mean apex diameter. The horizontal and vertical lines represent the diameter of the apical meristem which is the distance between the insertion of the outermost bract primordia measured in two directions at 90° to each other. (b) Stage 1. Broccoli apex at vegetative stage. The vegetative apex (A) was a small, pointed meristem surrounded by narrow, developing leaf primordia (L). (c) Stage 2. Broccoli apex at transitional stage. This stage was marked by a widening and flattening of the apical meristem (A). Bract primordia (B) were initiated around the apex. (d) Stage 3. Broccoli apex at early reproductive stage. Floral initiation was taken to have occurred at this stage with the development of first-order branch primordia (PB) in the axils of the bract primordia (B). (e) Stage 4. Broccoli apex at reproductive stage. Bract (B) and floral primordia (PB) were initiated distinctly in a spiral phyllotaxy. (f) Stage 5. Broccoli apex at reproductive stage. Numerous bract and floral primordia have developed. Second-order branch primordia (SB) were initiated from the apices of the first-order branch primordia (PB). Eventually, third and higher order branch primordia were initiated from their respective shoot apices. Horizontal bars represent 100 μm.
Table 4.1. Apex description during the transition from a vegetative to reproductive apex for broccoli.

<table>
<thead>
<tr>
<th>Morphological stage</th>
<th>Apex description</th>
<th>Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The apical meristem (A) was a small, pointed, shoot tip surrounded by developing foliage leaf primordia (L).</td>
<td>4.1b</td>
</tr>
<tr>
<td>2</td>
<td>Just before floral initiation, the dome-shaped apical meristem was widened and flattened and bract primordia (B) were initiated.</td>
<td>4.1c</td>
</tr>
<tr>
<td>3</td>
<td>Floral initiation was evident by the development of first-order floral branch primordia (PB). After this, only bract and floral primordia were initiated and no initiation of new foliage leaf primordia was observed.</td>
<td>4.1d</td>
</tr>
<tr>
<td>4</td>
<td>More bract and floral branch primordia were initiated in a spiral phyllotaxy and it was possible to distinctly observe this stage under low magnification (x40).</td>
<td>4.1e</td>
</tr>
<tr>
<td>5</td>
<td>Further development of numerous bract and floral primordia.</td>
<td>4.1f</td>
</tr>
<tr>
<td>6</td>
<td>Second-order floral branch primordia were initiated from the apices of the first-order branch primordia, which in turn initiated further orders of floral branch primordia. The elongation of some of the developing floral branch primordia preceded the transition to the floral phase.</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Floral branches elongated further and four sepals were initiated on the tips of the elongated floral branches.</td>
<td>-</td>
</tr>
</tbody>
</table>

4.3.2 Definition of the apex diameter at initiation

The apex diameter determined from the scanning electron micrographs showed significant differences between the morphological stages (P<0.01) (Table 4.2).

Table 4.2. Mean (± s.e.) apex diameter (μm) and range (μm) of broccoli at the five morphological stages during the transition from a vegetative to reproductive
apex measured from scanning electron micrographs. Mean apex diameter between the morphological stages were significantly different at P=0.01 using the nonparametric Kruskal-Wallis test of ranked diameter.

<table>
<thead>
<tr>
<th>Morphological stage</th>
<th>Mean apex diameter (µm)</th>
<th>Range (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92 ± 11</td>
<td>66-120</td>
</tr>
<tr>
<td>2</td>
<td>304 ± 34</td>
<td>241-375</td>
</tr>
<tr>
<td>3</td>
<td>513 ± 30</td>
<td>425-550</td>
</tr>
<tr>
<td>4</td>
<td>672 ± 12</td>
<td>650-700</td>
</tr>
<tr>
<td>5</td>
<td>1,212 ± 63</td>
<td>1090-1388</td>
</tr>
</tbody>
</table>

The ranges in the apex diameter of each stage did not overlap. Mean diameter was smallest during vegetative stage (Stage 1), and significantly increased as the development progressed to Stage 5. The mean apex diameter at floral initiation (Stage 3) was 513 ± 94 µm (mean ± 95% CI).

From the field experiment at Gatton College, the mean apex diameter at Stage 3 was 500 ± 3 µm (mean ± 95% CI) when measured using light microscopy. There were no significant effects (P>0.05) of photoperiod, sowing time and cultivar treatments on apex diameter at this stage, nor were there significant interactions.

### 4.4 Discussion

#### 4.4.1 Morphological development of the apical meristem

Stage 3 was selected as floral initiation because it was the stage at which a new structure (first order-floral branch primordia) first developed on a previously vegetative apex. This was consistent with the approach used by Salter (1969) and Moncur (1981) for the closely related cauliflower. The absence of new foliage leaf primordia in broccoli after the initiation of first-order floral branch primordia (PB) at Stage 3 was also reported by other authors, thereby indicating the determinant growth habit of broccoli (Diputado and Nichols 1989, Wurr et al. 1991a, 1992, Kieffer et al. 1998). At this stage, it was also possible to differentiate between leaves and bracts.
To determine the timing of floral initiation in a broccoli crop, the apices of three randomly selected plants can be removed every 3-days, dissected under a stereoscopic microscope and the morphological stage of the apex compared to the electron micrographs, until the apex rating has reached at least Stage 5 (Table 4.1, Plate 4.1f). The timing of floral initiation can then be determined by linear interpolation, when the graph of apex rating against time from emergence reaches Stage 3 (Tan et al. 1997).

It is interesting to compare these findings with reports for green sprouting broccoli (Gauss and Taylor 1969a), cauliflower (Sadik 1962, Salter 1969, Moncur 1981), radish (*Raphanus sativus* L.) (Hayward 1938, Moncur 1981) and brassica oilseed (*B. campestris* L. and *B. napus* L.) (Orr 1978, Moncur 1981, Polowick and Sawhney 1986, Smith and Scarisbrick 1990). This study found that the morphological development of broccoli meristem is similar to that of green sprouting broccoli and cauliflower. Bracts, which are not floral organs but small, modified leaves that subtend flowers or floral branches (Steeves and Sussex 1972) were also common in green sprouting broccoli and cauliflower but are rare in radish and brassica oilseed. The widening and flattening of the apical meristem in Stage 2 just prior to floral initiation, was also observed in green sprouting broccoli, cauliflower and cabbage (Thompson 1933), but a highly convex apex was observed in radish and brassica oilseed just prior to floral initiation. The development of higher-order floral branches also occurs in sprouting broccoli and cauliflower but not in radish and oilseed rape where the floral apex primordia elongates and differentiates into pedicel and floral bud without initiating higher-order floral branches. Apparently, the morphological development of broccoli and cauliflower apices during the transition from vegetative to reproductive stages are similar, but quite different from other brassicas such as radish and brassica oilseed.

4.4.2 Diameter of the apex at initiation
The mean apex diameter at Stage 3 of 513 ± 94 µm (n = 4) determined from electron micrographs was confirmed by the light microscopy study, although it showed a more precise mean apex diameter of 500 ± 3 µm due to the larger sample size (n = 144). The latter measurement of apex diameter at Stage 3 is similar to that reported by Wurr et al. (1995) who found that 50% of broccoli plants had reached floral initiation when the apex diameter was 490 ± 8 µm. However, an apex width of 259 µm was reported by Gauss and Taylor (1969a), for a stage which closely corresponded to Stage 3. The smaller apex may be due to their definition of apex width, which was the horizontal distance between the 2 uppermost leaf or floral branch primordia, whereas the apex diameter used in the present study included the floral branch primordia as in Wurr et al. (1991a, 1992).

The diameter of the apex during the transitional and reproductive stages was reported to be greater than 2000 µm by Fyffe and Titley (1989), which is approximately four times that found here and by Wurr et al. (1995). However, Fyffe and Titley (1989) used low magnification (×20) to view the apex (Fyffe 1987), and this could have reduced the accuracy of the measurements. Other workers have used higher magnifications (×100 or ×150).

The mean apex diameter for cauliflower at the time of floral initiation was defined as 600 µm by Salter (1969). The work reported here and that of Wurr et al. (1995) suggests that the mean apex diameter at initiation for broccoli is smaller, being closer to 500 µm.

### 4.5 Conclusions
The set of electron scanning micrographs of morphological development of the apical meristem of broccoli referred to as Stages 1 to 5, provided the basis for describing floral initiation. Floral initiation (Stage 3) was indicated by a widened and flattened apex along with appearance of first-order floral branch primordia. The micrographs provide a standard reference for field and laboratory assessment of floral initiation and should enhance broccoli research. Furthermore, light microscopy studies showed the mean apex diameter at floral initiation to be 500 µm which was independent of cultivar, sowing time and photoperiod. When the apex reaches 500 µm, floral initiation can be determined to have occurred.
Chapter 5
Freeze-induced reduction of broccoli yield and quality

5.1 Introduction

In the winters of 1994 and 1995 on the eastern Darling Downs, south-east Queensland, broccoli head yield and quality were downgraded after sub-zero temperatures were experienced (Jauncey, P. 1996 pers. comm.). Severe frosts (-7 to -8 °C air temperature) for three consecutive days in August 1995 resulted in growth retardation and reduction of head yield and quality. Ideally, broccoli plants growing during winter should not only survive but also resist freezing injury (Fuller et al. 1989). This chapter examines interactions between low temperature, crop development and crop yield and quality.

Varying levels of freezing tolerance are associated with different plant organs. In cabbage the relative freezing tolerance of organs is petiole < upper pith (stem) < middle pith < lamina < lower pith (Manley and Hummel 1996). Inner and outer petiole tissues were killed at –8 °C but pith and lamina tissues survived. At –12 °C only the lower pith survived without injury and at –16 °C all tissues were killed. It has been suggested (Fuller et al. 1994) that the cold hardiness mechanism in foliar portions of cauliflower is not expressed in the curd, since freezing always proved fatal with florets (epidermal region) having a flaccid and water-soaked appearance on thawing. Since cauliflower curd is incapable of surviving freezing per se, the only method of tolerance is freeze avoidance by supercooling. Exotherm detection studies showed a range of curd freezing points from –1 °C to –6.3 °C (Grout et al. 1982). Curd tissue, when frozen as isolated florets, supercooled over the range of –1 to –12 °C, and the overall mean freezing point of all curd tissue tested was –6.4 °C (Fuller et al. 1994).

Little is known about the relative freeze sensitivity of broccoli at different developmental stages. In many crops, the vegetative stage is thought to be the most tolerant, since the more frost-sensitive reproductive parts are not yet present. Freeze tests on cold-hardened
brassica oilseed (*B. napus* L. var. *oleifera*) seedlings at the 5-leaf stage showed that the killing temperature for 50% of the population (LT$_{50}$) was –9 to –10 °C (Kulesza et al. 1986). Sub-zero temperature treatments between –2 to –4 °C for 0–4 days did not induce apical abortion (‘blindness’) in broccoli cultivar, ‘Marathon’ (Forsyth et al. 1999). However, broccoli seedlings kept in a growth chamber at –3 to –5 °C for 16 or 34 days had high mortality levels (Miller et al. 1985). During floral initiation the apical meristem should be able to avoid freezing to a certain extent due to thermal insulation by wrapper-leaves. However, as the inflorescence enlarges, wrapper-leaves slowly unfold, exposing the young inflorescence to ambient temperatures (Tapsell et al. 1990). The ability of broccoli inflorescence to avoid freezing by supercooling is not known.

An important mechanism of freezing injury is secondary water stress, resulting from cytosolic dehydration as extracellular ice crystals grow (Li 1984). During extracellular-type freezing, air can be driven out of the intercellular space, resulting in the translucent appearance of frozen plant tissue. Upon thawing, plant tissues become limp with a water-soaked appearance. As ice in intercellular spaces melts the former air spaces are flooded and protoplasts lack turgor. If cells are not irreversibly damaged and thawing is very slow, intercellular water can be concomitantly re-absorbed. On the other hand, upon thawing freeze-killed cells are seemingly plasmolysed. Plasmolysis is attributable to the inability of dead and freely permeable protoplasts to re-absorb water. While the elastic cell wall can almost regain its original form, the dead protoplast will remain contracted giving the false appearance of plasmolysis (Levitt 1980, Li 1984).

The work described simulates natural frost events, where temperatures are reduced slowly, in order to test the resistance of broccoli plants to extracellular freezing. The objective was to describe and quantify the effect of sub-zero temperatures imposed at sequential broccoli plant development stages on leaf mortality, shoot apex mortality, and head yield and quality.
5.2 Materials and methods

5.2.1 Field-grown broccoli (Experiments 1 and 2)

Broccoli plants were obtained from a commercial farm at the Brookstead location. Details of the soil type at this location are described in Chapter 3 (Section 3.2). Cultivar ‘Fiesta’ (see Chapter 3, Section 3.1) was sown on 27 March 1998. Nutrients and water were supplied at non-limiting rates. Before sowing, 50 kg N and 100 kg P ha\(^{-1}\) were broadcast and incorporated into the prepared soil. A side-dressing of 155 kg N and 50 kg K ha\(^{-1}\) was applied 61 days after sowing. Plants were furrow irrigated regularly to prevent soil moisture deficit exceeding 20 mm. All insect pests and weeds were controlled rigorously.

Seedlings emerged 5 days after sowing and 50% floral initiation, using Stage 3 (Chapter 4) was recorded in late autumn (14 May 1998). Tissues for testing freeze injury were sampled from plants selected at random on 11 May 1998, during the late vegetative development stage (Experiment 1), and on 25 May 1998, 11 days after 50% floral initiation (Experiment 2). Leaf lamina and petiole tissues (3 replicates each) for Experiment 1 and leaf lamina and shoot apex tissues (5 replicates each) for Experiment 2 were evaluated for freezing sensitivity by relative electrical conductivity (REC) (see Section 5.2.4.b) and vital staining tests (see Section 5.2.4.c) after exposure to –7, -11, -15 or –19 °C and –5, -7, -9 or –11 °C, respectively for the durations given in Section 5.2.3.

5.2.2 Pot experiment (Experiment 3)

A completely randomised design was used for the pot experiment with five replicate plants for each treatment. This experiment was conducted at The University of Queensland, Gatton College plant nursery (latitude 27°33’S, longitude 152°20’E, altitude 89 m) in the Lockyer Valley approximately 80 km west of Brisbane, Queensland. Two cultivars, ‘Fiesta’ and ‘Marathon’, were sown into 265 mm diameter (8 L) plastic pots in
autumn (2 April 1998) and grown outdoors. The medium in each pot was composted sawdust : pinebark : sand (4:3:1 by volume). Fertilisers were 40 g slow release (3-4 month formulation) Osmocote® Plus (15% N ; 4.8% P ; 10.8% K plus trace elements; Scotts), 10 g dolomite, 10 g Azalon® (38% N), 10 g Saturaid® and 6 g coated iron (King and Morris 1994a). Also, foliar sprays of Flowfeed CO3® (20.6% N ; 8.5% P ; 15.9% K and trace elements; Grow Force) and calcium nitrate (15.5% N ; 19% Ca; Norsk Hydro Asa) (1.5 g L⁻¹) were applied once a week.

Seedlings emerged three days after sowing and 50% floral initiation was recorded on 3 and 5 June 1998 for ‘Marathon’ and ‘Fiesta’, respectively.

Plants were exposed to sub-zero temperatures for durations given in Section 5.2.3 at intervals of three weeks (see Fig. 5.1), coinciding with three developmental stages: 9-leaf stage (19 May), 15-leaf stage (9 June), and 22-leaf stage (30 June 1998). These were the vegetative, floral initiation and buttoning stages, respectively. The vegetative stage was treated at minimum temperatures of –5, -7, -9 and -11 °C in addition to the control (ambient temperature) for durations given in Section 5.2.3. Floral initiation and buttoning stages were treated at –1, -3, -5 and -7 °C and included a control. The –9 and –11 °C treatments were discarded as temperatures below –5 °C were lethal to the vegetative stage of pot-grown broccoli.

Insect pests and weeds were rigorously controlled. Irrigation was applied daily by overhead sprinklers.

5.2.3 Sub-zero temperature treatments

Field-grown tissue samples and whole pot-grown plants were exposed to sub-zero temperatures in a 3 m high x 2 m wide x 2 m deep freezer fitted with a compressor driven by a variable speed motor. Temperature was regulated with a Carel® IR32 digital thermostat controller. Controller accuracy was checked using type T copper/constantan
thermocouples and a calibrated 1200 series Squirrel® datalogger (Grant Instruments Ltd, UK). Thermocouple probes (0.32 mm diameter and 6 mm long) were inserted into leaf petioles. Temperatures were reduced at 0.2 °C min⁻¹ until –1 °C was reached. Hoar frost was simulated at –1 °C by spraying the plants with a fine mist of water and by fogging the chamber with carbon dioxide crystals from a fire extinguisher to nucleate ice crystal formation (Single and Fletcher 1978, Fletcher and Cullis 1988). After initiation of freezing, temperatures were reduced by 2 °C h⁻¹ to the pre-determined level and held constant for 1 h. Thawing was slowed by holding the tissues or plants at 4 °C for 2 h before opening the door to the chamber to allow the temperature to rise to ambient.

Pot-grown plants were transferred to a recovery greenhouse for 3 days before being placed in ambient conditions again.

5.2.4 Data collection

(a) Crop ontogeny

The time of emergence, floral initiation and buttoning were recorded as described in Chapter 3 (Section 3.3.2). All broccoli heads of each cultivar were harvested when 50% of the control plants of each cultivar reached an inflorescence diameter of 100 mm (Dufault 1996).

(b) Relative electrical conductivity (REC)

After whole plants had been treated in the freezer and fully thawed, five 10 mm diameter lamina disks, 5 mm long petioles or 2 mm long shoot apices were removed and placed into 10 mL distilled water in sealable 25 mm diameter glass vials (Fuller et al. 1989, Manley and Hummel 1996). Lamina disks were cut with a cork borer from fully expanded leaves. Electrical conductivity (EC) readings to measure electrolyte leakage were made at 20 °C using a TPS® Model WP-84 conductivity meter. After shaking the contents and standing at 20 °C for 30 min the EC of the eluate was measured to give the
initial reading. Tubes and their contents were then autoclaved at 120 °C and a pressure of 103 kPa for 15 min to give total electrolyte leakage. EC was measured again when solutions had cooled to 20 °C to give the final reading. The degree of damage caused by freezing was expressed as relative electrical conductivity (REC) (Dexter et al. 1932, Flint et al. 1967): \( \text{REC} = \frac{\text{Initial EC reading}}{\text{Final EC reading}} \). High REC indicates greater electrolyte leakage from tissue that has been damaged through freezing.

(c) Vital staining

The capacity of cells to reduce triphenyl tetrazolium chloride (TTC) is the basis of a vitality assay (Steponkus and Lanphear 1967, Li 1984). Cells which are not injured after a freeze-thaw cycle can still reduce the colourless form of TTC to the reddish formazan derivative. Tissue exposed to sub-zero temperatures was allowed to thaw, incubated at 4±2 °C for 48 h in petri dishes containing moistened filter paper, and then placed in individual glass vials containing 5 mL 0.5% (w/v) TTC in phosphate buffer (pH 7.3). The tissue was vacuum infiltrated at –33 kPa (Shorter and Joyce 1998) for 15 min and left in the dark for 10 h. Samples which did not stain red were considered dead (Manley and Hummel 1996).

(d) Shoot apex, leaf lamina and petiole mortality

Shoot apices were examined 21 days after the sub-zero temperature treatments and recorded as being alive or dead. Living shoot apices remained green and continued to grow, whereas killed shoot apices were brown. Leaf lamina and petiole mortalities were assessed 14 days after the sub-zero temperature treatments by estimating the amount of necrotic leaf area using a 5-point linear scale (0, no damage; 5, 100% damage) (Fuller et al. 1989). Leaf lamina and petiole mortalities were also converted to indices and analysed as binomial data. Lamina and petiole mortality indices were calculated based on the presence (score = 1) or absence (score = 0) of injury to a leaf lamina or petiole, respectively. Lamina and petiole destruction indices were calculated based on whether
complete (score = 1) or only some lamina and petiole mortality were present (score = 0), respectively (Fletcher and Cullis 1988).

(e) Yield and quality

‘Marathon’ and ‘Fiesta’ plants that survived sub-zero treatments and produced heads were harvested in late winter on 16 and 22 July 1998, respectively. The following yield measurements were made: head diameter – mean of two measurements taken 90° across the head (mm); head fresh weight – gravimetric determination of head mass (g); and head dry weight – weight after drying in a forced-draught oven at 90 °C to constant weight (g) (Chung 1985a).

Quality attribute assessments of head shape, branching angle, cluster separation, evenness of bud size, bud colour, bud size and hollow stem were made as described in Chapter 3 (Section 3.3.4).

(f) Ambient temperature

Daily maximum (data not presented) and minimum temperatures (°C) (Fig. 5.1) were obtained from a standard weather station located approximately 100 m from the Gatton College plant nursery. Temperatures for the commercial farm at Brookstead were obtained from an on-farm automatic weather station (Chapter 3, Fig. 5.1)
Fig. 5.1. Daily minimum temperatures (°C) from 31 March to 23 July 1998 at (O) Gatton College and (■) Brookstead in south-east Queensland. The time scale (day of the year) is in Julian days (Jday) where 1 = 1 Jan. Timing of sub-zero treatments for Experiment 1 (E1), Experiment 2 (E2), and the vegetative (E3V), floral initiation (E3F) and buttoning (E3B) stages of development in Experiment 3 are indicated.

5.2.5 Data analysis

Data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS Version 6.12 (SAS Institute 1989).

5.3 Results
5.3.1 Electrolyte leakage of field-grown broccoli (Experiments 1 & 2)

Leaf lamina tissue from field-grown broccoli explants sampled at the late vegetative stage appeared more susceptible than petioles to freezing injury in Experiment 1. Electrolyte leakage from lamina tissue was significantly higher (P<0.01) at sub-zero temperatures < -7 °C (Fig. 5.2a). This was confirmed by vital staining (data not presented), which showed dead lamina tissues at temperatures < -7 °C. After –11 °C treatment, TTC was reduced in the petiole tissue but not in the lamina tissue. REC of leaf lamina and shoot apices from explants sampled after the floral initiation stage suggest that the lethal temperature was between –7 °C and –9 °C for field-grown broccoli, since REC was low in this range (Fig. 5.2b). REC of lamina tissue was used as a freeze injury index in further experiments. It produced greater precision [l.s.d. (5%) = 0.03, P<0.001] between replicates of the same treatment compared to REC of shoot apices [l.s.d. (5%) = 0.19, P<0.01]. REC of lamina may also be a sensitive indicator of shoot apex mortality at floral initiation since electrolyte leakage of >50% (REC) was observed for both lamina and shoot apex tissue at –9 °C but not at –7 °C.
Fig. 5.2. Effect of sub-zero temperature treatments (°C) on relative electrical conductivity (REC) of (a) (●) leaf lamina and (■) petiole for explants sampled during the late vegetative stage (Experiment 1), and REC of (b) (♦) leaf lamina and (♦) shoot apices for explants sampled just after floral initiation (Experiment 2) from field-grown broccoli cultivar ‘Fiesta’ at Brookstead. The mean REC of controls (ambient temperature) were (a) 0.11 (lamina and petiole) for Experiment 1 and (b) 0.05 (lamina) and 0.31 (apex) for Experiment 2. Vertical lines indicate l.s.d. values at P=0.05 for sub-zero temperature treatments. Data presented in this figure are treatment means (n = 3 for a; n = 5 for b).
5.3.2 Mortality of pot-grown broccoli (Experiment 3)

The lethal temperature for the vegetative stage of potted plants was higher than –5 °C but it could not be determined accurately from REC of lamina electrolyte leakage tests as there were no higher temperature treatments for this stage of pot-grown broccoli (Fig. 5.3a). The lethal temperature for the floral initiation stage was between –3 °C and –5 °C and for the buttoning stage was between –3 °C and –7 °C (Fig. 5.3a). Thus, plants appeared to be slightly more resistant to sub-zero temperatures in later stages of development.

The leaf lamina mortality index showed that the lower temperature threshold for damage was –3 °C for floral initiation and buttoning stages (Table 5.1). There was no significant cultivar effect (P>0.05) (data not presented). Lamina mortality rating data showed significant interaction (P<0.05) between sub-zero temperature treatment, stage of development and cultivar. This was due to ‘Marathon’ showing slightly greater lamina damage than ‘Fiesta’ at –5 °C during the floral initiation stage (detailed data not presented). Since the cultivar effect was not visibly significant, cultivar means were pooled (n = 10) for lamina mortality rating (Fig. 5.3b).

At –3 °C minor leaf damage occurring as epidermal splitting (Plate 5.1a), similar to that observed in the field (Jauncey, P. 1996 pers. comm.), was recorded. At –5 °C chlorosis and severe necrosis of the leaf lamina (Plate. 5.1b) occurred. Lethal temperature for the floral initiation and buttoning stages was between –3 °C and –5 °C. Petiole mortality ratings showed that petiole tissue was damaged at lower temperatures than lamina tissue (Fig. 5.3c). There was no significant cultivar effect (P>0.05). The lethal temperature at floral initiation was between –3 °C and –7 °C, and at buttoning, between –5 °C and –7 °C.
Plate 5.1. Freezing injury symptoms in broccoli. (a) Minor leaf damage showing epidermal splitting caused by frosts (-6 °C) at Brookstead. (b) Severe necrosis and chlorosis of leaf lamina leaving the petiole relatively undamaged.
Fig. 5.3. Effect of sub-zero temperature treatment (ambient, -1, -3, -5, and –7 °C) and stage of development [(●) vegetative, (■) floral initiation and (♦) buttoning] on (a) relative electrical conductivity (REC) of leaf lamina, (b) leaf lamina mortality rating (1-5), and (c) leaf petiole mortality rating (1-5) for pot-grown broccoli at Gatton College (Experiment 3). The control (ambient temperature) means were (a) 0.07 for REC and 0 for both (b) leaf lamina and (c) petiole mortality ratings. Vertical lines indicate l.s.d. values at $P=0.05$ for sub-zero temperature treatment by stage of development interactions. Data presented in this figure are sub-zero temperature treatment by stage of development interaction means ($n = 10$). Means for the vegetative stage at –1 and –3 °C are not available as these treatments were not imposed.
The lethal temperature for shoot apices during floral initiation was between –3 °C and –5 °C and during buttoning was between –5 °C and –7 °C (Table 5.1). This was a definitive sensitivity index since once the shoot apex is dead the plant cannot produce marketable heads.

Table 5.1. Summary of sub-zero temperature treatments (ambient, -1, -3, -5, and –7 °C) and stage of development (floral initiation and buttoning) critical temperature ranges (°C) for pot-grown broccoli where damage or destruction was observed for >95% of the sample population in binomial vital staining, lamina mortality index, lamina destruction index, petiole mortality index, petiole destruction index or shoot apex destruction index data.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Critical temperature range (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Floral initiation</td>
</tr>
<tr>
<td>Vital staining</td>
<td>-3 to -5</td>
</tr>
<tr>
<td>Lamina mortality index</td>
<td>-1 to -3</td>
</tr>
<tr>
<td>Lamina destruction index</td>
<td>-3 to -5</td>
</tr>
<tr>
<td>Petiole mortality index</td>
<td>-3 to -7</td>
</tr>
<tr>
<td>Petiole destruction index</td>
<td>-5 to -7</td>
</tr>
<tr>
<td>Shoot apex destruction index</td>
<td>-3 to –5</td>
</tr>
</tbody>
</table>
5.3.3 Yield and quality of pot-grown broccoli (Experiment 3)

Head diameters for the –1 °C and –3 °C treatments at the floral initiation stage and the –5 °C treatment at the buttoning stage were significantly (P<0.05) smaller than for plants grown in ambient conditions (Table 5.2). There were no significant differences (P>0.05) in head diameters between cultivars and stage of development in the controls (ambient temperature) which confirmed that the time of harvest based on an inflorescence diameter of 100 mm in 50% of the control plants was an objective and representative harvest time. The fresh and dry weights of heads showed similar effects with the –1 °C and –3 °C treatments at the floral initiation stage, and the –5 °C treatment at the buttoning stage producing significantly lower head weights (P<0.05) than the controls.

Cluster separation, bud colour and evenness ratings were significantly lower (P<0.05) for the –5 °C treatment at the buttoning stage than in other treatments (Table 5.2), and there was no significant cultivar effect (P>0.05). Even though the growing shoot apices survived the –5 °C treatment, quality was reduced. Hollow stem was observed in all heads for the –5 °C treatment at the buttoning stage. The significant interaction (P<0.05) between sub-zero temperature treatment, stage of development and cultivar was mainly due to the –5 °C treatment at the buttoning stage for ‘Fiesta’ which showed a significant adverse change in head shape (Fig. 5.4a) and branching angle (Fig. 5.4b) ratings. Thus, ‘Fiesta’ was more susceptible to the –5 °C treatment than ‘Marathon’. Only significant cultivar main effects (P<0.01) were detected in bud size measurements. ‘Fiesta’ (2.14 ± 0.05 mm) had a significantly larger bud size than ‘Marathon’ (1.89 ± 0.05 mm) across all treatments.
Table 5.2. Effect of sub-zero temperature treatments (ambient, -1, -3, -5, and -7 °C) and stage of development (floral initiation and buttoning) on yield: head diameter (mm), head fresh weight (g) and head dry weight (g), and quality ratings: bud colour (1-5), bud evenness (1-5) and cluster separation (1-5) for pot-grown broccoli. Means followed by the same letter are not significantly different at P = 0.05 by Fisher’s protected l.s.d. test and conducted only when F-test probability was significant at $P \leq 0.05$. Data presented in this figure are sub-zero temperature treatment by stage of development interaction means (n = 10). Dash (-) indicates no quality ratings were made as plants were killed resulting in no yield.

<table>
<thead>
<tr>
<th>Stage of development (A)</th>
<th>Sub-zero temperature treatments (°C) (B)</th>
<th>Diameter (mm)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Colour rating (1-5)</th>
<th>Bud evenness rating (1-5)</th>
<th>Cluster separation rating (1-5)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Ambient</td>
<td>-1</td>
<td>-3</td>
<td>-5</td>
<td>-7</td>
<td>F-test probability</td>
<td>L.s.d (P = 0.05)</td>
</tr>
<tr>
<td>Floral initiation</td>
<td>105a</td>
<td>93 bc</td>
<td>87cd</td>
<td>0e</td>
<td>0e</td>
<td>P&lt;0.001</td>
<td>11.58</td>
</tr>
<tr>
<td>Buttoning</td>
<td>106a</td>
<td>104ab</td>
<td>103ab</td>
<td>76d</td>
<td>0e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floral initiation</td>
<td>190a</td>
<td>147bc</td>
<td>129c</td>
<td>0e</td>
<td>0e</td>
<td>P&lt;0.01</td>
<td>31.56</td>
</tr>
<tr>
<td>Buttoning</td>
<td>181a</td>
<td>172ab</td>
<td>166ab</td>
<td>81d</td>
<td>0e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floral initiation</td>
<td>19a</td>
<td>15b</td>
<td>13b</td>
<td>0d</td>
<td>0e</td>
<td>P&lt;0.001</td>
<td>2.95</td>
</tr>
<tr>
<td>Buttoning</td>
<td>18a</td>
<td>18a</td>
<td>18a</td>
<td>8d</td>
<td>0e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floral initiation</td>
<td>5a</td>
<td>5a</td>
<td>5a</td>
<td>-</td>
<td>-</td>
<td>P&lt;0.001</td>
<td>0.32</td>
</tr>
<tr>
<td>Buttoning</td>
<td>5a</td>
<td>5a</td>
<td>4.8a</td>
<td>4.3b</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floral initiation</td>
<td>4.7a</td>
<td>4.6a</td>
<td>4.7a</td>
<td>-</td>
<td>-</td>
<td>P&lt;0.001</td>
<td>0.48</td>
</tr>
<tr>
<td>Buttoning</td>
<td>4.8a</td>
<td>4.9a</td>
<td>4.1b</td>
<td>2.6c</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floral initiation</td>
<td>4.6a</td>
<td>4.9a</td>
<td>4.7a</td>
<td>-</td>
<td>-</td>
<td>P&lt;0.05</td>
<td>0.59</td>
</tr>
<tr>
<td>Buttoning</td>
<td>4.9a</td>
<td>4.8a</td>
<td>4.5a</td>
<td>3.9b</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 5.4. Effect of sub-zero temperature treatment (ambient, -1, -3, -5, and –7 °C), stage of development [(■) floral initiation and (♦) buttoning] and cultivar ['Fiesta' (closed symbols), and ‘Marathon’ (open symbols)] on (a) head shape rating and (b) branching angle rating for pot-grown broccoli at Gatton College (Experiment 3). The control (ambient temperature) means were (a) 4.9 for head shape, and (b) 4.4 for branching angle rating. Vertical lines indicate l.s.d. values at P=0.05 for sub-zero temperature treatment, stage of development by cultivar interaction. The data presented in this figure are sub-zero temperature treatment, stage of development by cultivar interaction means (n = 5). Means for floral initiation at –5 °C were not available as the shoot apex of plants were killed, producing no yield.
5.4 Discussion

5.4.1 Yield and quality reduction

The vegetative stage was sensitive to sub-zero temperatures although no temperature treatments higher than –5 °C were imposed. The floral initiation stage appeared to be most sensitive to freezing injury as yields (fresh and dry head weights) were significantly reduced at –1 °C and –3 °C and the shoot apices were killed at –5 °C resulting in no yield. No significant apical abortion was reported in ‘Marathon’ seedlings treated with sub-zero temperatures of –2 to –4 °C for 0–4 days (Forsyth et al. 1999). However, temperatures ≤ –5 °C were used in the present study, which were lower than the range used by Forsyth et al. (1999).

There was no significant yield reduction at the buttoning stage for treatments at –1 °C and –3 °C and the shoot apices survived the –5 °C treatment, but produced very poor quality heads. Plants could have become hardened (cold acclimatised) in later stages of development (Kohn and Levitt 1965). The uneven bud size and poor cluster separation in plants treated at –5 °C during the buttoning stage could have resulted from site specific developmental arrest (Bjorkman and Pearson 1998) which can occur during floral bud development and internode elongation (Carr and Irish 1997). More mature buds that are enlarging when the freezing stress is applied may continue to do so, resulting in a large size difference between affected buds and those only a few spaces lower in the phyllotactic (arrangement of buds in an inflorescence) spiral. Subsequent production of additional buds results in many small, stunted buds at the centre of each paracleade (axillary flowering shoot) being evident as bud unevenness. In a normal head these would be so few as to be covered by more mature buds giving a highly uniform appearance (Bjorkman and Pearson 1998).
Pot-grown plants treated during floral initiation stage at –1 °C and –3 °C and during the buttoning stage at –5 °C produced significantly smaller and lower weight heads. Their growth appeared to be retarded after the sub-zero temperature treatment, possibly due to impairment of chloroplast functioning (Bauer et al. 1992, 1994). Photosynthesis would be substantially reduced after freezing due to disturbance of both chloroplastic processes and stomatal opening. This suggests that crop development models based only on simple thermal time (see Chapters 7 and 8) without restrictions may not apply after severe frosts have caused freezing injury, since a ‘shock’ or ‘stress’ over-rides normal developmental processes.

5.4.2 Tissue damage

Leaf lamina mortality ratings suggested that ‘Marathon’ lamina is more sensitive to sub-zero temperatures (-5 °C) than ‘Fiesta’ at the floral initiation stage. However, no yield was obtained from either cultivars as the –5 °C treatment resulted in shoot apex mortality. The only significant cultivar interaction found at harvest were related to head shape and branching angle (i.e. quality) ratings for ‘Fiesta,’ which were significantly lower than ‘Marathon’ following the –5 °C treatment at buttoning stage. In brassica oilseeds (B. juncea L. Coss & Czern and B. napus var. oleifera) leaf injury estimates are believed to be of limited relevance because the reduction in leaf area has a limited direct effect on yield (Dhawan et al. 1983, Lardon and Triboi-Blondel 1995). However, results presented here show that, although there were only subtle cultivar differences in head quality, measurements such as REC and lamina and shoot apex mortality provide a good indication of whether satisfactory broccoli yield and quality can be attained.

REC and mortality ratings suggested that petiole tissue may be more resistant to freezing injury than lamina tissue (Plate 5.1b). This is in contrast to cabbage leaf lamina tissue which was reported to be more frost resistant than cabbage petiole tissue, based on work done with excised tissue (Manley and Hummel 1996). However, results in the present study were based on whole plant freezing and are considered more realistic. The
relatively large thermal mass (viz. low surface area to volume ratio) in petioles compared
to lamina could have acted as heat storage, thereby delaying freezing of petioles (Tapsell
et al. 1990, Simons, D. 1998, pers. comm.). Whole plants were used in this study since
farmers are more interested in the effects of sub-zero temperatures on crops, and these
data are more relevant to commercial production than that for excised tissue.

5.4.3 Cold acclimation

Data from the pot experiment (Experiment 3) suggested that the lethal temperature for
broccoli was between −3 °C and −5 °C, while that from the field experiments
(Experiments 1 & 2) suggested that the lethal temperature was between −7 °C and −9 °C.
This latter range is similar to that reported for freezing tests on cold hardened brassica
oilseed (B. napus var. oleifera) seedlings at the 5-leaf stage, which had a LT$_{50}$ at −9 to
−10 °C (Kulesza et al. 1986). The difference between the pot and field data may be due
to cold acclimation. Potted broccoli were grown at Gatton College where temperatures
were always above 0 °C (Fig. 5.1), and, in any case automatic sprinklers were set to
operate if sub-zero temperatures occurred. Field-grown broccoli plants experienced 17
days with < 2 °C daily minimum air temperatures at Brookstead on the Darling Downs
(Fig. 5.1). Maximum hardening at +3 °C of greenhouse grown cabbage seedlings
resulted in a freeze killing point of −7 to −10 °C. However, cabbage seedlings grown in
growth chambers (25/15 °C day/night) and hardened for 6 weeks at successively lower
temperatures from +5 °C to −3 °C attained a freeze killing point of −20 °C (Kohn and
Levitt 1965). It thus appears that field-grown broccoli at Brookstead were more cold
acclimated and therefore could tolerate lower sub-zero temperatures than pot-grown
broccoli at Gatton.

With a view to achieving cold acclimation by molecular techniques, the CBF1
transcription factor driving COR (cold-regulated) genes associated with cold acclimation
has been engineered in Arabidopsis thaliana (L.) Heynh. (Jaglo-Ottosen et al. 1998).
Broccoli is closely related to *Arabidopsis* (Carr and Irish 1997) and belongs to the same family Brassicaceae. It may thus prove possible to breed broccoli with enhanced freeze resistance in the future (Sigareva and Earle 1997).

### 5.5 Conclusions

The floral initiation stage was most sensitive to freezing injury as broccoli head yield and quality were significantly reduced at sub-lethal temperatures of −1 °C and −3 °C and the shoot apices were killed at −5 °C resulting in no yield. There was no significant yield reduction at the buttoning stage for treatments of −1 °C and −3 °C and the shoot apices survived the −5 °C treatment but produced very poor quality heads possibly due to developmental arrest. Lethal temperature for pot-grown broccoli was between −3 °C and −5 °C whereas the lethal temperature for field-grown broccoli was between −7 °C and −9 °C, suggesting acclimation of the plants growing at the colder field site. These studies show that exposure to sub-zero temperatures can reduce not only the yield but also the quality of broccoli.
Chapter 6

Influence of temperature and photoperiod
on broccoli yield and quality

6.1 Introduction

Yield of marketable heads is an important factor for broccoli growers, and sowing date can have a major impact on marketable yield. Sowing schedules based on optimum yield, quality and time to maturity have been developed in south-east Queensland for export to south-east Asia from May to September (Titley 1985, 1987). Plant size and marketable yield of ten cultivars for once-over harvest broccoli in north-west Tasmania were reduced when sowings were delayed from January to March (Chung and Strickland 1986). Yields below 5 t ha⁻¹ head fresh weight were obtained in March to May sowings harvested in winter compared with yields above 10 t ha⁻¹ for December and January sowings. Lower yields and smaller head sizes, caused by low temperatures inducing early head development and reducing plant growth rate, were reported for late autumn sowings harvested in late winter in New South Wales (Murison 1983) and Scotland (Thompson and Taylor 1970).

There is limited information on the effect of temperature on broccoli quality. Curd abnormalities due to high and low temperature include descriptions such as ‘blindness’, ‘fuzziness’, ‘bractiness’ and ‘riciness’ (Fujime 1983, Fujime and Okuda 1996, Wurr et al. 1996). Studies have indicated that heat stress (35 °C) did not affect broccoli at the vegetative stage but may be critical during the time the immature inflorescence (buttoning) measures 5 to 10 mm in diameter (Heather et al. 1992). However other results, using more precise morphological methods and a larger sample size, showed that apical meristems were affected only if heat (35 °C) occurred much earlier, during floral initiation when the meristem was less than 1 mm wide (Bjorkman and Pearson 1998, Tan et al. 1998). Polynomial equations relating growing season mean (GSM) temperatures to quality attributes such as head shape, colour, cluster separation, bractiness and bud size have been proposed from work in
South Carolina, USA (Dufault 1996). Sub-zero temperatures can severely reduce broccoli head yield and quality, and retard plant growth (Chapter 5, Tan et al. 1999).

Researchers have speculated that photoperiod may affect broccoli yield and quality but no data were presented (Chung 1985a, Chung and Strickland 1986, Thompson and Taylor 1970). Research in the UK showed no evidence for photoperiod sensitivity in the closely related cauliflower (Thapa 1994, Hadley and Pearson 1998). Broccoli plants grown in a controlled environment at 17 °C, under long-day treatment (16 h light), formed floral primordia one week earlier than those under a short-day treatment (8 h light) (Fujime et al. 1988). However, many researchers working with field-grown broccoli in the UK have assumed that crop development does not respond to photoperiod (Marshall and Thompson 1987a, Wurr et al. 1991a, 1992, 1995). Field studies are needed to determine the response of broccoli yield and quality to photoperiod.

Better understanding of genotype and environmental interactions will help farmers optimise yield and quality, by matching cultivars to time of sowing. The objective of this study was to quantify the temperature and photoperiod responses of yield and quality attributes for three commercial broccoli cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) grown under field conditions in a sub-tropical environment. By using natural and extended photoperiods, and a range of sowing dates, yield and quality data under a broad range of temperature and photoperiod conditions were collected and analysed.

6.2 Materials and methods

6.2.1 Quality attributes of commercial grades

To determine the quality attributes of domestic and export grades of broccoli, attributes of head fresh weight, diameter, shape, branching angle and cluster separation (described in Chapter 3, Section 3.3.4) were first obtained from a commercial packing house at the Brookstead location (described in Chapter 3, Section 3.2). These quality attributes of commercial grades were later used to make comparisons with the quality attributes from the field experiment with photoperiod
extension (see Section 6.2.2). Hence, it will be possible to determine whether broccoli quality in the field experiment will meet market requirements. The packing house had quality assurance accreditation for exporting broccoli to Japan and south-east Asia. Broccoli was graded twice in this commercial operation. In the commercial crop that was harvested by hand, the first step in grading occurred when immature, over-mature or poor quality heads were not harvested. At the packing house, the commercial crop was pre-cooled in a cold room, then further graded by skilled packers into cartons destined for specific markets (Tan et al. 1997).

A randomised complete block design with four replicate broccoli heads was used for the analysis. There were five grades (‘Japan’, ‘SE Asia’, ‘Chain’, ‘Central L’ and ‘Central S’) of broccoli (see Table 6.1), each classified according to market destination; one for export to Japan (‘Japan’), another to south-east Asia (‘SE Asia’), and three for the domestic market. For the domestic market, higher quality produce were sent to the chain stores (for example, Coles, Woolworths) and supermarkets (‘Chain’) while poorer quality produce were separated into large size (‘Central L’) and small size (‘Central S’) categories and sent to Central Wholesale Markets. Broccoli for each grade was randomly sampled from polystyrene cartons packed by eight different experienced packers during the 1996 broccoli season. Yield and quality attributes were recorded. All the broccoli grades packed and sampled were accepted by the respective markets. The highest prices were obtained from the export (‘Japan’ and ‘SE Asia’) and ‘Chain’ grades.

6.2.2 Field experiment with photoperiod extension

A field experiment with photoperiod extension was conducted at the University of Queensland, Gatton College. Full details of the experimental design are presented in Chapter 3 (Section 3.1).

6.2.3 Data collection
Daily maximum and minimum temperatures (°C), crop data, including dates of emergence, floral initiation and harvest maturity were recorded as described in Chapter 3 (Section 3.3). For each sowing date and cultivar, the daily minimum and maximum temperatures were averaged from emergence to harvest and defined as minimum and maximum growing season mean (GSM) temperatures (Dufault 1996). The same temperatures were averaged from floral initiation to harvest and defined as floral season mean (FSM) temperatures.

The following yield measurements were made: head diameter – mean of two measurements taken 90° across the head (mm); head fresh weight – gravimetric determination of head mass (g); and head dry weight – weight after drying in a forced-draught oven at 90 °C to constant mass (g) (Chung 1985a). Vegetation (bracts, leaves and stem) were cut off at the cotyledon scars and weighed separately. Dry weights of both head and vegetation were recorded for sowings #4 to #8 only.

Fresh weight harvest index (FWHI) (Chung 1982, Shelp 1988, Jett et al. 1995) (described in Chapter 2, Section 2.3.1, Equation 2.1) was defined as:

\[ \text{FWHI} = \frac{100 \times \text{head fresh weight}}{\text{head fresh weight} + \text{vegetation fresh weight}} \]  \hspace{1cm} (6.1)

Dry weight harvest index (DWHI) was defined as:

\[ \text{DWHI} = \frac{100 \times \text{head dry weight}}{\text{head dry weight} + \text{vegetation dry weight}} \]  \hspace{1cm} (6.2)

Fresh and dry weights of tops were defined as the total plant (sum of head and vegetation) fresh and dry weights, respectively. Although both fresh and dry weights were measured, only detailed fresh weight data are presented herein, since broccoli is a fresh product and both fresh and dry weights showed similar responses. Head quality attribute assessments of head shape, branching angle, cluster separation, evenness of bud size, bud colour, bud size, bractiness and hollow stem, as described in Chapter 3 (Section 3.3.4), were made.

6.2.4 Data analysis
Analysis of variance (ANOVA) was completed for yield and quality attributes to test the independent and interactive effects of photoperiod extension, sowing date and cultivar, using the general linear model (GLM) procedure of SAS version 6.12 (Little 1985, SAS Institute 1989, Section 3.4 in Chapter 3). Linear, broken linear and polynomial regression were performed to investigate relationships between GSM and FSM minimum and maximum temperatures, and the yield and quality attributes. The most biologically meaningful regression with a high F-test value was selected from these options (Dufault 1996). Multivariate analysis on quality rating data (evenness of bud size, bud colour, head shape, branching angle and cluster separation) was carried out using principal components analysis (eigenanalysis) of SAS (Manly 1986). Analysis of variance was also conducted for the first two principal components similar to the approach used for evaluation of lettuce cultivars (Tan 1991).

6.3 Results

6.3.1 Quality attributes of commercial grades

‘Japan’, ‘Chain’ and ‘Central L’ grades had greater (P<0.01) head fresh weights and diameters than ‘SE Asia’ and ‘Central S’ (Table 6.1). Both export grades (‘Japan’ and ‘SE Asia’) had higher (P<0.01) branching angle and cluster separation ratings than the central market grades (‘Central L’ and ‘Central S’) (Table 6.1).

| Table 6.1. Head yield and quality attributes of five broccoli grades (Export Japan, Export South-east Asia, Domestic Chain Stores, Domestic Central Markets Large and Small), expressed as head fresh weight (g head⁻¹), head diameter (mm), head shape (1-5), branching angle (1-5) and cluster separation (1-5) ratings packed by eight packers in a packing house near Brookstead, south-east Queensland. Means of grades are averaged over eight packers (n = 40). |
L.s.d. values are at P=0.05 using Fisher’s protected l.s.d. tests for grade main effect. Means followed by the same letter within the same row are not significantly different at P=0.05.

<table>
<thead>
<tr>
<th>Quality attribute</th>
<th>Export grade</th>
<th>Domestic grade</th>
<th>l.s.d. (P = 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Japan (L)</td>
<td>SE Asia (S)</td>
<td>Chain (L)</td>
</tr>
<tr>
<td>Fresh weight (g head⁻¹)</td>
<td>248b</td>
<td>214c</td>
<td>290a</td>
</tr>
<tr>
<td>Head diameter (mm)</td>
<td>112b</td>
<td>93e</td>
<td>107c</td>
</tr>
<tr>
<td>Head shape (1-5)</td>
<td>4.75a</td>
<td>4.47ab</td>
<td>4.62a</td>
</tr>
<tr>
<td>Branching angle (1-5)</td>
<td>3.97a</td>
<td>4.19a</td>
<td>3.34b</td>
</tr>
<tr>
<td>Cluster separation (1-5)</td>
<td>4.60a</td>
<td>4.16b</td>
<td>3.91bc</td>
</tr>
</tbody>
</table>

6.3.2 Yield

Fresh and dry head weights were constant (225 ± 4.5 g head⁻¹; 24 ± 0.5 g head⁻¹, respectively; P>0.05) across photoperiod, sowing dates and cultivars since broccoli were harvested at a standard stage of development, when 50% of the head diameters were ≥ 100 mm. Fresh weights (Fig. 6.1a) and dry weights (detailed data not presented) of tops decreased (P<0.01) with later sowings. A broken linear regression indicated a relationship (P<0.01) between fresh weight of tops and GSM minimum temperature (Fig. 6.1b). There was a significant increase (P<0.01) in both FWHI (Fig. 6.1c) and DWHI (data not presented) with later sowings. A broken linear relationship (P<0.01) was found between FWHI and GSM minimum temperature (Fig. 6.1d). As GSM minimum temperature decreased, fresh weight of tops decreased linearly while FWHI increased linearly (since head fresh weights were constant). However, there was no definite relationship between fresh weight of tops or FWHI, and GSM minimum temperatures ≥ 10 °C.
Fig. 6.1. Effect of sowing date [Julian day (Jday) where 1 = 1 Jan] (closed symbols), growing season mean (GSM) minimum temperature (open symbols) and broccoli cultivar ['Fiesta' (circles), 'Greenbelt' (squares) and 'Marathon' (triangles)] on (a and b) fresh weight of tops (g), and (c and d) fresh weight harvest index (%) for eight sowing dates and two photoperiods (natural and 16 h) in a field experiment at Gatton College. The data presented in (a) and (c) are sowing date by cultivar interaction means (n = 6), and in (b) and (d) are experimental treatment means averaged over three blocks (n = 3). Vertical lines in (a) and (c) indicate l.s.d. values at P=0.05 (for comparisons between sowing date) for sowing date by cultivar interaction.
6.3.3 Quality

Quality attributes were determined by cultivar rather than the environment (Table 6.2). ‘Fiesta’ had higher (P<0.01) head shape and branching angle ratings than ‘Greenbelt’ and ‘Marathon’ and bud evenness ratings for ‘Fiesta’ and ‘Greenbelt’ were higher (P<0.01) than for ‘Marathon’. Bud size in ‘Fiesta’ was larger (P<0.01) than in ‘Greenbelt’ and ‘Marathon’. Although bractiness was higher (P<0.01) in ‘Fiesta’, its incidence was too low to affect quality. Except for the last three sowings (#6, #7, and #8), ‘Marathon’, had lower (P<0.05) cluster separation and colour ratings than ‘Fiesta’ and ‘Greenbelt’ (Fig. 6.2a and 6.2b). Bud colour rating of ‘Marathon’ was acceptable at GSM minimum temperatures < 8 °C and increased linearly (Fig. 6.2c) with decreasing GSM minimum temperatures < 10 °C. At GSM minimum temperatures ≥ 10 °C, there was no definite relationship between colour rating and GSM minimum temperature. A broken linear equation described the relationship between cluster separation rating and GSM minimum temperature (Fig. 6.2d). The coefficient of determination of this equation explained only 50% of the variation. However, the relationship was stronger at GSM minimum temperatures < 8 °C. There was no definite relationship between cluster separation rating and GSM temperatures ≥ 8 °C.
Table 6.2. Head quality attributes of three broccoli cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’), expressed as head shape (1-5), branching angle (1-5), cluster separation (1-5) and bud evenness (1-5) ratings, bud size (mm), bractiness (number of bracts protruding through head), percentage head dry weight (%) (dry/fresh weight), and principal components 1 and 2 (PC1 & PC2) grown under a range of photoperiod and temperature regimes at Gatton College, south-east Queensland. Means of cultivars are averaged over two photoperiods (natural and 16 h) and eight sowing dates (n = 48). L.s.d values are at P=0.05, using Fisher’s protected L.s.d. tests for the cultivar main effect. Means followed by the same letter within the same row are not significantly different at P=0.05.

<table>
<thead>
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<th>Quality attribute</th>
<th>Cultivar</th>
<th>L.s.d. (P = 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>‘Fiesta’</td>
<td>‘Greenbelt’</td>
</tr>
<tr>
<td>Head shape (1-5)</td>
<td>4.67a</td>
<td>4.23b</td>
</tr>
<tr>
<td>Branching angle (1-5)</td>
<td>4.23a</td>
<td>3.56c</td>
</tr>
<tr>
<td>Bud evenness (1-5)</td>
<td>4.67a</td>
<td>4.60a</td>
</tr>
<tr>
<td>Bud size (mm)</td>
<td>2.31a</td>
<td>2.06b</td>
</tr>
<tr>
<td>Bractiness</td>
<td>0.16a</td>
<td>0.02b</td>
</tr>
<tr>
<td>% dry weight (head)*</td>
<td>11.71a</td>
<td>11.28b</td>
</tr>
<tr>
<td>Principal component 1</td>
<td>1.05a</td>
<td>-0.07b</td>
</tr>
<tr>
<td>Principal component 2</td>
<td>0.08a</td>
<td>0.66b</td>
</tr>
</tbody>
</table>

* Means of cultivars are averaged over two photoperiods (natural and 16 h) and four sowing dates (n = 24).
The eigenvalue for a principal component indicates the variance that it accounts for out of a total of five. The first principal component accounted for 55% (eigenvalue of 2.77) of the variation, while the second principal component only accounted for 21% (eigenvalue of 1.05). The first two principal components were considered since these were the only ones with eigenvalues > 1 (Manly 1986). The first principal component (PC₁) was:
PC\(_1\) = 0.390X_1 + 0.245X_2 + 0.527X_3 + 0.484X_4 + 0.535X_5

where X\(_1\) – evenness of bud size; X\(_2\) – bud colour; X\(_3\) – head shape; X\(_4\) – branching angle; X\(_5\) – cluster separation; and X\(_1\) to X\(_5\) are standardised variables. Coefficients of PC\(_1\) (eigenvectors) were all positive, indicating that PC\(_1\) is an index of broccoli quality. A high PC\(_1\) value can be associated with a high quality broccoli while a low PC\(_1\) value can be associated with an unmarketable broccoli.

The second principal component (PC\(_2\)) was:

PC\(_2\) = 0.267X_1 + 0.803X_2 – 0.305X_3 – 0.420X_4 + 0.120X_5

The low coefficient of X\(_5\) means that the value of this variable (cluster separation) had little effect on PC\(_2\). There appears to be a contrast between X\(_1\) (evenness of bud size) and X\(_2\) (bud colour) on one hand, and X\(_3\) (head shape) and X\(_4\) (branching angle) on the other. PC\(_2\) will be high if X\(_1\) and X\(_2\) are high, or X\(_3\) and X\(_4\) are low. The converse is also true. Hence, PC\(_2\) represents a certain “quality” difference between the treatments. The first two principal components accounted for 76% of the variation in the data.

When PC\(_1\) and PC\(_2\) were plotted (Fig. 6.3), most of ‘Fiesta’ were grouped with high values of PC\(_1\). The majority of ‘Greenbelt’ had high PC\(_2\) values while most of ‘Marathon’ had low PC\(_2\) values. Analysis of variance of PC\(_1\) and PC\(_2\) showed that PC\(_1\) of ‘Fiesta’ was higher (P<0.01) than that of ‘Greenbelt’ and ‘Marathon’, and PC\(_2\) of ‘Greenbelt’ was higher (P<0.01) than that of ‘Marathon’. There were no significant differences (P>0.05) between sowing dates.
Fig. 6.3. Plot of broccoli cultivars ['Fiesta' (●), 'Greenbelt' (□) and ‘Marathon’ (△)] against the first two principal components (PC1, PC2) for eight sowing dates and two photoperiods (natural and 16 h) in a field experiment at Gatton College.

6.3.4 Photoperiod

Photoperiod did not have any significant effect (P>0.05) on head yield or quality in this study.
6.4 Discussion

6.4.1 Yield

Fresh weight of tops decreased as sowings progressed from early to late autumn, which is consistent with other findings (Thompson and Taylor 1970, Murison 1983, Chung and Strickland 1986). However, FWHI and DWHI increased with later sowings. Progressively lower GSM minimum temperatures during winter may have retarded vegetative growth (Fig. 6.1b) and increased partitioning into the reproductive head (Fig. 6.1d), which agrees with the results of Gauss and Taylor (1969b) and Kage and Stutzel (1999). The proportion of dry matter partitioned into the heads (DWHI) in this study varied from 19 – 26% which was much higher than the 9 – 16% reported in New Zealand (Diputado and Nichols 1989). A probable explanation was that the lower density (Chung 1982, 1985a) sowings (0.35 m by 0.75 m) in New Zealand produced top dry weights of 117 – 305 g plant$^{-1}$ (Diputado and Nichols 1989) compared with 93 – 144 g plant$^{-1}$ in this study. The larger plants had reduced DWHI since head dry weights were similar in both studies.

6.4.2 Quality

Quality attributes of head shape, branching angle, bud size and evenness, and bractiness varied by cultivar, with environment contributing relatively little to quality. Branching angle rating of all three cultivars exceeded 3.3 and fitted the criteria for both export (‘Japan’ & ‘SE Asia’) and ‘Chain’ grades. Only head shape rating of ‘Fiesta’ exceeded 4.4 to fit the criteria for both export (‘Japan’ & ‘SE Asia’) and ‘Chain’ grades. ‘Fiesta’ was the best overall cultivar when assessed on the quality attributes of head shape and branching angle, followed by ‘Greenbelt’ and ‘Marathon’. ‘Marathon’ was the inferior cultivar as early sowings reduced its bud colour and cluster separation ratings (Figs. 6.2a and 6.2b), and cluster separation rating of ‘Marathon’ only exceeded 3.9 to fit the criteria for both export (‘Japan’ and ‘SE Asia’) and ‘Chain’ grades in the last two sowings.
A broken linear relationship was fitted between both bud colour and cluster separation, and GSM minimum temperature in this study. This was similar to cultivars, ‘Packman’ and ‘Southern Comet’ which were also affected by GSM minimum temperature although third order polynomial equations were fitted (Dufault 1996). The broken linear models in this study for ‘Marathon’ fit the available data best and are more biologically meaningful than the third order polynomials used by Dufault (1996). The models in the present study suggest that as GSM minimum temperature increased, a negative linear model may be applied until a threshold of 8-10 °C was reached. Thereafter, no clear trend was evident.

The quality index (PC1) summarised the results, showing that ‘Fiesta’ produced consistently better quality heads than ‘Greenbelt’ and ‘Marathon’. ‘Greenbelt’ was distinctly separated from ‘Marathon’ by PC2 because of the contrast in bud characteristics such as evenness and colour, and head form characteristics such as head shape and branching angle. ‘Greenbelt’ was grouped with high values of PC2 since it had good bud characteristics but poor head form characteristics, while ‘Marathon’ was grouped with low values of PC2 since it had poor bud characteristics but good head form characteristics. In this study, principal components analysis showed that overall quality was affected more by genotype than environment. Hence, this analysis can be a useful tool for evaluating cultivar and environment interactions in broccoli quality.

### 6.4.3 Photoperiod

Photoperiod did not affect broccoli yield and quality in this study. This is consistent with recent work in the UK which showed no evidence for photoperiod sensitivity in three cultivars of cauliflower. Future work should be directed at studying the effect of temperature and perhaps, solar radiation (Klaring 1998, Kage and Stutzel 1999) on yield and quality. There was no evidence to justify suggestions by other researchers (Chung 1985a, Chung and Strickland 1986, Thompson and Taylor 1970) that broccoli yield and quality may be affected by photoperiod.
6.5 Conclusions

Fresh and dry weight harvest indices increased as sowings were delayed from early to late autumn. Broken linear relationships were found between fresh weight of tops, fresh weight harvest index, and GSM minimum temperature. Quality attributes were influenced more by genotype than environment. Overall, ‘Fiesta’ was the best performing cultivar, with higher head shape and branching angle ratings than ‘Greenbelt’ and ‘Marathon’. Cluster separation and bud colour of ‘Marathon’ were only acceptable for export when grown under GSM minimum temperatures < 8 °C. Photoperiod did not affect yield and quality in this study.
Chapter 7

Broccoli development is predominantly determined by temperature

7.1 Introduction

Development of predictive models for broccoli ontogeny will be useful for farmers. Better quantification of the impact of climatic variability on broccoli development would allow producers to alter management decisions, including marketing arrangements. This would improve the reliability of supply and ultimately increase farmers’ incomes and reduce financial risks. Models of crop development are also useful in determining the timing of cultural practices, such as fertiliser application, irrigation and crop protection that are related to crop ontogeny (Theunissen and Sins 1984).

An approach to estimating floral initiation is to predict vernalisation requirements. A vernalisation model to predict change in apex diameter with temperature from transplanting to floral initiation for broccoli has been developed under controlled environment conditions (Wurr et al. 1995). However, broccoli plants are receptive to vernalisation only after the juvenile stage (Fontes et al. 1967, Miller et al. 1985, Fujime 1988). Furthermore, juvenility is a poorly understood phenomenon. No quantitative relationships that predict cessation of juvenility have been developed, beyond the requirement for production of a similar number of leaves (4 leaves > 2 cm) (Wiebe 1990). Due to the difficulty of predicting the end of the juvenile stage, thermal time models have been widely used to predict floral initiation (Diputado and Nichols 1989, Fyffe and Titley 1989, Pearson et al. 1994, Hadley and Pearson 1998).

The temperature response for crop development is widely defined in terms of three cardinal temperatures: base ($T_{\text{base}}$), optimum ($T_{\text{opt}}$) and maximum ($T_{\text{max}}$). $T_{\text{base}}$ and $T_{\text{max}}$ are the temperatures below and above which the plant does not develop, while $T_{\text{opt}}$ is the temperature at which development proceeds most rapidly (Birch et al. 1998a). Existing models that use the same temperature responses from sowing to
harvest (Chung 1981, Titley 1987, Marshall and Thompson 1987a, 1987b, Dufault 1997) do not predict important crop development stages such as floral initiation. Errors introduced into a linear thermal time model by an incorrect $T_{\text{base}}$ may be generalised by saying that when $T_{\text{base}}$ is too high, chronological time for floral initiation and harvest maturity is over-predicted at a cooler site and under-predicted at a warmer site, and if $T_{\text{base}}$ is too low, the reverse occurs (Arnold 1959). Critiques of the thermal time approach also point out that the cardinal temperatures might have to change depending on phenological stage (Wang 1960, Marshall and Thompson 1987b).

There are variations in the reported cardinal temperatures from sowing to floral initiation. Some researchers used a standard $T_{\text{base}}$ of 4.5 °C for all cultivars (Fyffe and Titley 1989) while others calculated a $T_{\text{base}}$ of 1 °C with a $T_{\text{opt}}$ of 21 °C using the least coefficient of variation method (Diputado and Nichols 1989).

There are also differences in the reported cardinal temperatures from floral initiation to harvest maturity. Reports of $T_{\text{base}}$ range from 0 °C (Wurr et al. 1991a, 1992, Greven 1998) to 3 °C (Diputado and Nichols 1989). A much higher $T_{\text{base}}$ of 7 °C has been reported for the cultivar, ‘Mercedes’ (Pearson and Hadley 1988), and for four cultivars from sowing to harvest (Dufault 1997). $T_{\text{opt}}$ and $T_{\text{max}}$ have received much less attention than $T_{\text{base}}$, presumably because most studies have been carried out in temperate environments. The only $T_{\text{opt}}$ reported were 15 °C (Wurr et al. 1991a, 1992, Wurr and Scaife 1992) and 17 °C (Greven 1998), and $T_{\text{max}}$ was 26.7 °C for the duration from sowing to harvest (Dufault 1997).

Broccoli plants respond to higher temperatures during the vegetative stage by increasing total number of leaves present at floral initiation. When temperature was increased from 13 °C to 29 °C, cultivar ‘Coastal’ formed buttons (immature inflorescences approximately 10 mm diameter) at 74 days from sowing, and the number of leaves initiated increased from 17 to 27 (Gauss and Taylor 1969b). Similarly, with the same cultivar, leaf number was increased from 18 to 24 as temperature increased from 13 °C to 29 °C (Wiebe 1975).
Seedlings of cultivars ‘Gem’ and ‘Bravo’ exposed to 14 or more days of cold temperatures (-5 °C to 2 °C) 13 or more days after sowing flowered earlier and had fewer nodes than plants not exposed to low temperatures (Miller et al. 1985).

Research on the effects of temperature has often assumed photoperiod does not modify floral initiation or flowering responses in broccoli (Miller et al. 1985, Marshall and Thompson 1987a, 1987b, Thapa 1994, Hadley and Pearson 1998). Effects of temperatures (17 to 23 °C) and photoperiods (8 h and 16 h light, light intensity not reported) on floral development was investigated in Japan (Kagawa 1965, Fujime et al. 1988). Plants under a long-day treatment (16 h) formed floral primordia one week earlier than those under a short-day treatment (8 h) at 17 °C. Long-day treatment also resulted in lower total leaf number at floral initiation than short-day (8 h) treatment at the same temperature (17 °C). A highly significant interaction of temperature with photoperiod (86-95 J s⁻¹ m⁻² light intensity, equivalent to 3.7 - 4.1 MJ m⁻² day⁻¹ for a 12 h day) for cultivar ‘Coastal’ (Gauss and Taylor 1969b) was probably due to the effect of increasing energy levels and not to photoperiod per se. Solar radiation was incorporated into thermal time models by some researchers (Marshall and Thompson 1987a, 1987b, Wurr et al. 1991a, 1992, Mourao and Hadley 1998), but other workers claim that solar radiation had no effect on crop development (Pearson and Hadley 1988, Fujime and Okuda 1994).

The objective of this study was to quantify the temperature and photoperiod response of three broccoli cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) from emergence to floral initiation (EFI), and from floral initiation to harvest maturity (FIHM). Data for development and leaf number under a broad range of temperature and photoperiod conditions in a sub-tropical environment were collected using natural and extended photoperiods, and a range of sowing dates. An iterative optimisation technique was applied to the data to derive $T_{\text{base}}$, $T_{\text{opt}}$, and photoperiod sensitivity for the three cultivars, and the results for $T_{\text{base}}$ and $T_{\text{opt}}$ were incorporated into thermal time models. These models were successfully tested on five cultivars (‘Fiesta’, ‘Greenbelt’, ‘Marathon’, ‘CMS Liberty’ and ‘Triathlon’) grown commercially on the Darling Downs during two years.
7.2 Materials and methods

7.2.1 Field experiment with photoperiod extension

A field experiment with photoperiod extension was conducted at the University of Queensland, Gatton College. Details of the experimental details and design are described in Chapter 3 (Section 3.1)

7.2.2 Commercial farm crops for testing the model

Crop ontogeny data to test the EFI and FIHM thermal time models were obtained from a commercial farm at the Brookstead location. The cultural practices and soil type in this location are described in Chapter 3 (Section 3.2). There were 60 sowings of 5 cultivars (‘Fiesta’, ‘Greenbelt’, ‘Marathon’, ‘CMS Liberty’ and ‘Triathlon’) over 2 growing seasons (1997 and 1998). Seeds were sown in double rows 0.25 by 0.25 m (8 plants m\(^{-2}\)) on beds 1.0 m apart. ‘Fiesta’ (35 sowings) was sown on 26 February, 3, 7, 14, 24 March, 1, 7, 11, 14, 21, 29 April, 2, 13, 15, 23, 29 May and 2 June 1997, and 25, 26 February, 4, 11, 18, 25, 27 March, 1, 3, 6, 14, 20, 21 April, 1, 2, 13, 14, 27 May 1998. ‘Greenbelt’ (7 sowings) was sown on 24, 28 February, 5, 8, 12, 20, 29 March 1997 and ‘Marathon’ (8 sowings) was sown on 17, 26 March, 4, 16, 24 April, 12, 23 May 1997 and 30 March 1998. Newly released cultivars, including the cytoplasmic male sterile, ‘CMS Liberty’ (Petoseed, USA) was sown 6 times on 27 February, 2, 9, 13, 16, 23 March 1998, and ‘Triathlon’ (Sakata, USA) was sown 4 times on 20, 21, 29, 30 April 1998.

7.2.3 Data collection

Daily maximum and minimum temperatures (°C), total daily solar radiation (MJ m\(^{-2}\)), leaf number, dates of emergence, floral initiation and harvest maturity were recorded as described in Chapter 3 (Section 3.3).

7.2.4 Data analysis
Analysis of variance (ANOVA) was completed to test the independent and interactive effects of photoperiod extension, sowing date and cultivar on chronological time, thermal time, and solar radiation (accumulated daily radiation) duration of EFI and FIHM, using the general linear model (GLM) procedure of SAS as described in Chapter 3 (Section 3.4). Pearson correlation coefficients between accumulated solar radiation and thermal time were determined using SAS to test the independence of the predictors.

An optimisation program, DEVEL (Holzworth and Hammer 1992), was used to determine the temperature and photoperiod responses of each cultivar from the experimental data for duration of EFI and FIHM. DEVEL contains a library of temperature and photoperiod functions which can be used separately or in combination to examine the independent and interactive effects of temperature and photoperiod. A simplex optimisation method is used by DEVEL. This requires starting conditions (estimates of the parameters to be optimised) to be supplied. Numerous initial conditions were used to guard against the identification of local optima, and to assess whether they converged to the same global optimised values (Devlin 1994). The 2-stage broken linear response best explained both the temperature (Fig. 1.3a) and photoperiod (Fig. 1.3b) responses (Chapter 1).

Accumulated thermal time (°C d) (Arnold 1959) for duration of EFI and FIHM (days, \(i = 1\) to \(n\)) were calculated using the optimised \(T_{\text{base}}\) and \(T_{\text{opt}}\) of 0 and 20 °C (described in this study) based on the equation (see Chapter 2, Section 2.7.2.c):

\[
\text{Thermal time} = \sum_{i=1}^{n} \left[ \frac{(T_{\text{Dmax}} + T_{\text{Dmin}})}{2} - T_{\text{base}} \right]
\]

(7.1)

where \(T_{\text{Dmax}}\) = maximum temperature for the day, \(T_{\text{Dmin}}\) = minimum temperature for the day. All \(T_{\text{Dmin}} < T_{\text{base}}\) were considered to be equal to 0 °C, and all \(T_{\text{Dmax}} > T_{\text{opt}}\) were considered to be equal to 20 °C (Barger System) (Arnold 1974, Titley 1985, 1987, Wurr et al. 1991a). Effective thermal time (ETT) (Scaife et al. 1987, Wurr and Fellows 1998) for each day was calculated from the following equation (See Chapter 2, Section 2.7.8, Equation 2.7):
1/ETT = 1/TT + a/R \hspace{1cm} (7.2)

where TT = thermal time (°C d) for the day, R = total solar radiation (MJ m$^2$) for the day, and $a$ = a unitless constant defining the relative importance of solar radiation and temperature for the cultivar concerned. The optimum value of $a$ for each cultivar was obtained by minimising the coefficient of variation among ETT of experimental units for each cultivar. The best attribute (chronological time, thermal time, accumulated solar radiation duration or ETT) for predicting FIHM was determined by the attribute that minimised the sowing date F value for each cultivar. The attribute should ideally be independent of sowing date.

7.3 Results

7.3.1 Photoperiod, cultivar and sowing date effects

Effects of photoperiod extension, cultivar, sowing date and their interactions on chronological time, thermal time [(°C d, calculated using $T_{base}$ and $T_{opt}$ of 0 and 20 °C, derived in this study (see Section 7.3.2)] and accumulated solar radiation (MJ m$^2$) for the duration of EFI, and total leaf number were analysed by analysis of variance (Table 7.1). The most notable interaction was between cultivar and sowing date which was significantly different (P<0.01) for both chronological time (Fig. 7.1a) and total leaf number (Fig. 7.1b). All cultivars showed a general trend of longer chronological duration of EFI when they were sown later in autumn (except for #8). For ‘Greenbelt’ and ‘Marathon’, total leaf number decreased with later sowings while for ‘Fiesta’, total leaf number decreased initially (#1 to #4) but became relatively constant in later sowings (#4 to #8).

Table 7.1. Main and interactive effects of photoperiod extension (PP), sowing date (SD) and cultivar (CV) on the chronological time (days), thermal time (°C d) and accumulated solar radiation (MJ m$^2$) during the interval from emergence to floral initiation, and total leaf number in broccoli [**, *, n.s. for P<0.01,
P<0.05, not significantly different (P=0.05) respectively]. Dash (-) indicates no l.s.d. was calculated as the F-test was not significant at P=0.05.

<table>
<thead>
<tr>
<th>Effect</th>
<th>PP</th>
<th>SD</th>
<th>CV</th>
<th>PP X SD</th>
<th>PP X CV</th>
<th>SD X CV</th>
<th>PP X SD X CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronological time</td>
<td>n.s.</td>
<td>**</td>
<td>*</td>
<td>**</td>
<td>**</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>l.s.d. (P=0.05)</td>
<td>-</td>
<td>0.86</td>
<td>0.47</td>
<td>1.26</td>
<td>0.78</td>
<td>1.39</td>
<td>-</td>
</tr>
<tr>
<td>Thermal time</td>
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<td>**</td>
<td>*</td>
<td>**</td>
<td>**</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>l.s.d.(P=0.05)</td>
<td>-</td>
<td>12.31</td>
<td>6.77</td>
<td>17.60</td>
<td>10.34</td>
<td>19.90</td>
<td>-</td>
</tr>
<tr>
<td>Solar radiation</td>
<td>n.s.</td>
<td>**</td>
<td>*</td>
<td>**</td>
<td>**</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>l.s.d. (P=0.05)</td>
<td>-</td>
<td>10.42</td>
<td>5.65</td>
<td>15.05</td>
<td>8.99</td>
<td>16.70</td>
<td>-</td>
</tr>
<tr>
<td>Total leaf no.</td>
<td>n.s.</td>
<td>**</td>
<td>*</td>
<td>n.s.</td>
<td>n.s.</td>
<td>**</td>
<td>n.s.</td>
</tr>
<tr>
<td>l.s.d. (P=0.05)</td>
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<td>0.62</td>
<td>0.26</td>
<td>-</td>
<td>-</td>
<td>0.86</td>
<td>-</td>
</tr>
</tbody>
</table>
Fig. 7.1. Effect of sowing date [Julian day (Jday) where 1 = 1 Jan] and cultivar ['Fiesta' (●), ‘Greenbelt’ (■) and ‘Marathon’ (♦)] on (a) chronological time (days) and (b) total leaf number from emergence to floral initiation (EFI) for eight sowing dates in a field experiment at Gatton College. The data presented are sowing date by cultivar interaction means (n = 6). Vertical lines indicate l.s.d. values at P=0.05 (for comparisons between sowing dates) for sowing date by cultivar interaction. Effect of (c) sowing date and photoperiod [natural (●) and 16 h (■)] on chronological time (days) from emergence to floral initiation (EFI) averaged over three cultivars of broccoli for eight sowing dates in a field experiment at Gatton College. Vertical line indicates l.s.d. value at P=0.05 (for comparisons between sowing dates) for sowing date by photoperiod interaction. The data presented are sowing date by photoperiod interaction means (n = 9).
Both ‘Fiesta’ and ‘Marathon’ had a significant (P<0.05) delay of one day in floral initiation in the extended photoperiod treatment compared with the control (data not presented). No significant photoperiod effect (P>0.05) was detected with ‘Greenbelt’. ‘Fiesta’ had a significantly (P<0.01) greater chronological duration of EFI than ‘Greenbelt’ and ‘Marathon’. The extended photoperiod treatment resulted in a delay (P<0.05) of one day in floral initiation for all cultivars in #5 and #6 only (Fig. 7.1c). Delayed floral initiation response to increased photoperiod suggests a very slight short-day photoperiod response in broccoli. Further analysis using DEVEL was carried out to explain these responses.

Effects of photoperiod extension, cultivar, sowing date and their interactions on chronological time, thermal time, accumulated solar radiation and ETT duration of FIHM were also analysed by analysis of variance (Table 7.2). Photoperiod extension did not affect the duration of FIHM when assessed by DEVEL, though significant responses were found by analysis of variance (Table 7.2). These significant responses are confounded by different environmental conditions affecting the duration of EFI. Since results of the DEVEL analysis were unaffected by this confounding factor (Birch 1996), they were used in further analyses.

### 7.3.2 Temperature response

$T_{\text{max}}$ was not determined for duration of both EFI and FIHM for any cultivar as the range of temperatures experienced during the autumn and winter growing period were relatively narrow. There were few days with high temperatures (e.g. $>25\, ^{\circ}\text{C}$), and thus determination of $T_{\text{max}}$ was not possible. Typically, thermal time models have four parameters consisting of thermal time calculated from cardinal temperatures of $T_{\text{base}}$, $T_{\text{opt}}$ and $T_{\text{max}}$. Since $T_{\text{max}}$ was not determined, EFI and FIHM thermal time models in this study have three parameters; viz. (i) thermal time calculated from (ii) $T_{\text{base}}$ and (iii) $T_{\text{opt}}$ for the duration of EFI and FIHM, respectively (see Fig. 1.3a in Chapter 1, Section 1.4).
Table 7.2. Main and interactive effects of photoperiod extension (PP), sowing date (SD) and cultivar (CV) on the chronological time (days), thermal time (°C d), accumulated solar radiation (MJ m⁻²), and effective thermal time (ETT) during the interval from floral initiation to harvest maturity (FIHM) in broccoli [**, *, n.s. for P<0.01, P<0.05, not significantly different (P=0.05) respectively]. Dash (-) indicates no l.s.d. was calculated as the F-test was not significant at P=0.05.

<table>
<thead>
<tr>
<th>Effect</th>
<th>PP</th>
<th>SD</th>
<th>CV</th>
<th>PP X SD</th>
<th>PP X CV</th>
<th>SD X CV</th>
<th>PP X SD X CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronological time</td>
<td>n.s.</td>
<td>**</td>
<td>**</td>
<td>n.s.</td>
<td>**</td>
<td>n.s.</td>
<td>**</td>
</tr>
<tr>
<td>l.s.d. (P=0.05)</td>
<td>-</td>
<td>2.10</td>
<td>0.78</td>
<td>-</td>
<td>-</td>
<td>2.76</td>
<td>3.11</td>
</tr>
<tr>
<td>Thermal time</td>
<td>n.s.</td>
<td>**</td>
<td>**</td>
<td>n.s.</td>
<td>n.s.</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>l.s.d. (P=0.05)</td>
<td>-</td>
<td>27.13</td>
<td>10.67</td>
<td>-</td>
<td>-</td>
<td>36.64</td>
<td>42.67</td>
</tr>
<tr>
<td>Solar radiation</td>
<td>n.s.</td>
<td>**</td>
<td>**</td>
<td>n.s.</td>
<td>n.s.</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>l.s.d. (P=0.05)</td>
<td>-</td>
<td>28.88</td>
<td>11.27</td>
<td>-</td>
<td>-</td>
<td>38.87</td>
<td>45.07</td>
</tr>
<tr>
<td>ETT</td>
<td>n.s.</td>
<td>**</td>
<td>**</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>*</td>
</tr>
<tr>
<td>l.s.d. (P=0.05)</td>
<td>-</td>
<td>24.52</td>
<td>9.45</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>37.81</td>
</tr>
</tbody>
</table>

T_base and T_opt of 0 and 20 °C were found for all three cultivars, these temperatures falling within the 10% confidence interval for the individual cultivars for duration of both EFI and FIHM (Tables 7.3 and 7.4). Although T_base and T_opt were consistent across cultivars for both EFI and FIHM, the thermal time requirement was specific to each cultivar. Thermal time duration of EFI for ‘Fiesta’, ‘Greenbelt’, and ‘Marathon’ were 670, 612, and 627 °C d respectively. This thermal time model explained 89%, 70% and 53% of the variation during EFI for ‘Fiesta’, ‘Greenbelt’ and ‘Marathon’ respectively (Table 7.3). Thermal time duration of FIHM for ‘Fiesta’, ‘Greenbelt’, and ‘Marathon’ were 664, 660, and 678 °Cd respectively for all sowing dates (Table 7.4). This thermal time model explained 90%, 80% and 36% of the variation during FIHM for ‘Fiesta’, ‘Greenbelt’ and ‘Marathon’ respectively.
Table 7.3. Optimum rate of development [expressed as rate of progress (day\(^{-1}\)), and chronological time (days)] and thermal time (TT °C d, mean ± s.e.) duration with optimised base and optimum temperatures of 0 and 20 °C during the time from emergence to floral initiation for three broccoli cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) grown under a range of photoperiod and temperature regimes in a field experiment at Gatton College, south-east Queensland.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Optimum rate of development (day(^{-1}))</th>
<th>10% confidence interval</th>
<th>Minimum chronological duration (days)</th>
<th>TT(°Cd) (mean ± s.e.)</th>
<th>(r^2) (^a)</th>
<th>(n) (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Fiesta’</td>
<td>0.026</td>
<td>0.026-0.026</td>
<td>38</td>
<td>670 ± 3.36</td>
<td>0.89</td>
<td>16</td>
</tr>
<tr>
<td>‘Greenbelt’</td>
<td>0.028</td>
<td>0.027-0.028</td>
<td>36</td>
<td>612 ± 5.43</td>
<td>0.70</td>
<td>16</td>
</tr>
<tr>
<td>‘Marathon’</td>
<td>0.026</td>
<td>0.026-0.026</td>
<td>38</td>
<td>627 ± 5.90</td>
<td>0.53</td>
<td>16</td>
</tr>
</tbody>
</table>

\(^a\) Coefficient of determination for the EFI thermal time model from DEVEL analysis.

\(^b\) Number of observations

‘Marathon’ had a consistently poor fit to the thermal time models due to greater within-cultivar variation since ‘Marathon’ had a higher coefficient of variation in thermal time than other cultivars. Thermal time for duration of EFI for ‘Fiesta’ was greater (P<0.01) than for ‘Marathon’, which was, in turn, greater (P<0.01) than for ‘Greenbelt’ (Tables 7.1 and 7.3). Thermal time duration of FIHM for both ‘Fiesta’ and ‘Greenbelt’ were not significantly different (P>0.05) from each other (Tables 7.2 and 7.4), but thermal time duration for ‘Marathon’ was slightly greater (P<0.05).
Table 7.4. Duration (mean ± s.e.) from floral initiation to harvest maturity, expressed as chronological time (days), thermal time (°C d), accumulated solar radiation (MJ m⁻²), and effective thermal time (ETT) for three broccoli cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) grown under a range of photoperiod and temperature regimes in a field experiment at Gatton College, south-east Queensland, as estimated from optimisation routines using DEVEL. Thermal time was calculated using base and optimum temperatures of 0 and 20 °C. ETT was calculated using α values of 0.045, 0.039 and 0.354 for ‘Fiesta’, ‘Greenbelt’ and ‘Marathon’. Means given are of 48 experimental units.

<table>
<thead>
<tr>
<th>Duration</th>
<th>Cultivar</th>
<th>‘Fiesta’ Mean ± s.e.</th>
<th>F valueα</th>
<th>‘Greenbelt’ Mean ± s.e.</th>
<th>F valueα</th>
<th>‘Marathon’ Mean ± s.e.</th>
<th>F valueα</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronological time</td>
<td>‘Fiesta’</td>
<td>49 ± 0.62</td>
<td>11.1</td>
<td>49 ± 0.62</td>
<td>22.6</td>
<td>50 ± 0.56</td>
<td>10.45</td>
</tr>
<tr>
<td></td>
<td>‘Greenbelt’</td>
<td>664 ± 5.50</td>
<td>1.5</td>
<td>660 ± 5.05</td>
<td>4.2</td>
<td>678 ± 6.96</td>
<td>7.73</td>
</tr>
<tr>
<td></td>
<td>‘Marathon’</td>
<td>619 ± 12.60</td>
<td>26.5</td>
<td>601 ± 10.97</td>
<td>49.0</td>
<td>617 ± 9.54</td>
<td>22.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>633 ± 5.21</td>
<td>1.3</td>
<td>633 ± 4.82</td>
<td>4.1</td>
<td>487 ± 4.45</td>
<td>3.85</td>
</tr>
</tbody>
</table>

α F value for sowing date for each cultivar. In this assessment, a low F value indicates constant value of the appropriate attribute over sowing dates.

7.3.3 Photoperiod response during EFI

Inclusion of photoperiod in analyses using DEVEL only accounted for an additional 6%, 2% and 4% of the variation for ‘Fiesta’, ‘Greenbelt’ and ‘Marathon’, respectively in EFI, calculated from the difference in coefficient of determination for the combined temperature and photoperiod model, and the temperature model only. There was no evidence from the analyses that the ceiling photoperiod was exceeded by the maximum photoperiod of 16 h. Analyses using DEVEL indicated that a critical photoperiod of 11 h could be appropriate. However, this must be used as an interim figure, as there were no data for photoperiods < 11 h. Even so, it is realistic to accept this value for south-east Queensland and probably other areas where photoperiods < 11 h do not occur. Due to the very low photoperiod sensitivity detected, the effect of photoperiod was ignored in further analyses. The limited photoperiod response was confirmed by the lack of any effect of extending photoperiod on total leaf number in all sowings (see Table 7.1).
7.3.4 Total solar radiation

Incorporation of solar radiation in the calculation of ETT did not improve the thermal time model for duration of EFI. In fact, precision was not improved, even when the parameter $a$ (which measures the sensitivity to solar radiation) was set at a very low value of 0.0001 (data not presented). This suggests that broccoli development is not sensitive to solar radiation during EFI, within the range of solar radiation received in these experiments. The low Pearson correlation coefficient (0.24, $n = 144$) between the predictors, accumulated solar radiation and thermal time indicates that these two predictors were largely independent in this study.

Since photoperiod extension did not have a significant effect, an attribute for predicting FIHM for each cultivar with the least F value between the sowing dates was investigated (Table 7.4). For this assessment, a low F value, indicating constant value of the appropriate attribute over sowing dates was used. Although ETT (Fig. 7.2d) predicted FIHM of each cultivar among the sowing dates with a lower F value than either chronological time (Fig. 7.2a) or accumulated solar radiation (Fig. 7.2c), F values were almost the same as for thermal time in ‘Fiesta’ and ‘Greenbelt’, and only marginally lower than for thermal time in ‘Marathon’ (Fig. 7.2b, Table 7.4). The thermal time model would describe duration of FIHM adequately since ETT only reduced F values marginally.

7.3.5 Total leaf number

There was a significant linear relationship ($P<0.01$) between total leaf number and average temperature for each cultivar (Fig. 7.3). There was a significant linear relationship ($P<0.01$) between thermal time duration of EFI and total leaf number for ‘Greenbelt’ and ‘Marathon’ but not ‘Fiesta’ ($P>0.05$) (Fig. 7.3). There was no significant effect ($P>0.05$) of photoperiod on total leaf number (Table 7.1).
Fig. 7.2. Effect of sowing date [Julian day (Jday) where 1 = 1 Jan] and cultivar ['Fiesta' (●), ‘Greenbelt’ (■) and ‘Marathon’ (♦)] on (a) chronological time (days), (b) thermal time (°C d), (c) accumulated solar radiation (MJ m⁻²), and (d) effective thermal time (ETT) duration for eight sowing dates in a field experiment at Gatton College, south-east Queensland. Vertical lines indicate l.s.d. values at P=0.05 (for comparisons between sowing dates) for sowing date by cultivar interaction for (a), (b) and (c), and l.s.d. value at P=0.05 for cultivar main effect for (d). The data presented are sowing date by cultivar interaction means (n = 6). No significant (P=0.05) sowing date by cultivar interaction was observed in (d).
Fig. 7.3. Effect of average temperature (°C) (open symbols) and thermal time (°C d) (closed symbols) from emergence to floral initiation (EFI) on the total leaf number at floral initiation in three broccoli cultivars, ‘Fiesta’ [circles (a,b)], ‘Greenbelt’ [squares (c,d)], and ‘Marathon’ [triangles (e,f)] for eight sowing dates in a field experiment at Gatton College. The data presented in this figure are experimental treatment means averaged over three blocks (n = 3).

7.3.6 Accuracy of fitted values from optimised $T_{base}$ and $T_{opt}$

Chronological duration of EFI (Fig. 7.4a) and FIHM (Fig. 7.5a) were fitted from the optimised temperature parameters derived using DEVEL. The goodness of fit of the fitted values for EFI for the three cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’)
from optimised values shows that most points fall very close to the 1:1 line (Fig. 7.4a). The RMSD for the pooled data was 1.6 days, and the regression (Fig. 7.4a) shows a good fit for chronological duration of EFI ranging from 39 to 48 days.

The goodness of fit of the fitted values for FIHM for the three cultivars from the optimised values shows that most points fall close to the 1:1 line (Fig. 7.5a) although the fit was not as good as that for EFI. The regression (Fig. 7.5a) shows a satisfactory fit for chronological duration of FIHM ranging from 40 to 54 days and RMSD for the pooled data was 1.9 days.

7.3.7 Evaluation of model against independent farm data

The model developed from the experimental data at Gatton College was then applied to the independent farm data at Brookstead (Fig. 7.4b and 7.5b). Coefficient of determination for predicted values of chronological duration of EFI from independent farm data using optimised temperature coefficients, accounted for 64% of the variation in observed data for the pooled analysis with five cultivars (‘Fiesta’, ‘Greenbelt’, ‘Marathon’, ‘CMS Liberty’ and ‘Triathlon’) (Fig. 7.4b). Predicted values fall close to the 1:1 line and RMSD for the pooled data was 1.8 days, representing approximately 4% of overall mean EFI. Hence, satisfactory prediction is provided for the chronological duration of EFI ranging from 40 to 55 days, although there was a tendency for over predictions at duration < 47 days and under predictions at duration > 47 days.

Coefficient of determination for predictions of chronological duration of FIHM from independent farm data using optimised temperature coefficients, accounted for 80% of the observed data for the pooled analysis using all five cultivars (Fig. 7.5b). Predicted values fall close to the 1:1 line and RMSD for the pooled data was 2.9 days, representing approximately 5% of overall mean FIHM, which provided good prediction for the chronological duration of FIHM ranging from 44 to 64 days.
Fig. 7.4. (a) Comparison between fitted and observed duration (days) from emergence to floral initiation for three broccoli cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) when grown at Gatton College; and (b) comparison between predicted and observed duration (days) from emergence to floral initiation for independent data from five broccoli cultivars (‘Fiesta’, ‘Greenbelt’, ‘Marathon’, ‘CMS Liberty’, and ‘Triathlon’) grown on a commercial farm in Brookstead in 1997 and 1998. Predicted duration based on thermal time was calculated using base and optimum temperatures of 0 and 20 °C, respectively, derived using the optimisation routine, DEVEL, from a field experiment at Gatton College, southeast Queensland.
Fig. 7.5. (a) Comparison between fitted and observed duration (days) from floral initiation to harvest maturity for three broccoli cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) when grown at Gatton College; and (b) comparison between predicted and observed duration (days) from floral initiation to harvest maturity of independent data from five broccoli cultivars (‘Fiesta’, ‘Greenbelt’, ‘Marathon’, ‘CMS Liberty’, and ‘Triathlon’) grown on a commercial farm in Brookstead in 1997 and 1998. Predicted duration based on thermal time was calculated using base and optimum temperatures of 0 and 20 °C, respectively, derived using the optimisation routine, DEVEL from a field experiment at Gatton College, south-east Queensland.
7.4 Discussion

7.4.1 Optimised temperature coefficients

The $T_{\text{base}}$ and $T_{\text{opt}}$ of 0 and 20 °C for the duration of EFI determined from the present study are consistent with a thermal time study in New Zealand (Diputado and Nichols 1989) where $T_{\text{base}}$ and $T_{\text{opt}}$ of 1 and 21°C respectively, were calculated using the least coefficient of variation method. However, a limited range of environments (e.g. no photoperiod treatment) was sampled in the New Zealand study, whereas the present study involved the development of 48 crops at Gatton College in a range of temperature and photoperiod environments generated over eight sowing dates. The optimised $T_{\text{base}}$ of 0 °C also agrees with other results (Marshall and Thompson 1987a, Grevsen and Olesen 1994). The model in this study explained 53% (for ‘Marathon’) to 89% (for ‘Fiesta’) of the EFI variation. This is better than the thermal time models developed for cauliflower from transplanting to floral initiation which explained only 40% to 60% of the variation (Grevsen and Olesen 1994).

The optimised $T_{\text{base}}$ and $T_{\text{opt}}$ of 0 and 20 °C for the duration of FIHM is generally consistent with most literature sources and is similar to optimised temperatures for duration of EFI. The optimised $T_{\text{base}}$ of 0 °C also agrees with other results (Marshall and Thompson 1987a, Wurr et al. 1991a, 1992, Grevsen 1998), but is lower than the $T_{\text{base}}$ of 3 °C reported (Diputado and Nichols 1989). There appears to be no need to use different $T_{\text{base}}$ for the various developmental intervals. No support was found in the present study for a higher $T_{\text{base}}$ of 7 °C as reported by Pearson and Hadley (1988) and Dufault (1997). The $T_{\text{opt}}$ of 20 °C in this study is higher than the values of 15 and 17 °C reported for temperate broccoli cultivars growing in the UK (Wurr et al. 1991a) and Denmark (Grevsen 1998), respectively.

Similar results with $T_{\text{base}}$ and $T_{\text{opt}}$ of 0 and 21 °C, respectively, were observed for all cultivars using the Barger System (Arnold 1974) thermal time method (Tittley 1985). Estimates of days to floral initiation and harvest maturity based on mean temperatures have been reported to be more accurate than thermal time estimates (Fyffe and Tittley...
but in coming to this conclusion, these authors assumed $T_{\text{base}}$ of 4.5 °C (Barger system) derived for sweet corn (Arnold 1974). This approach could have caused chronological time for floral initiation and harvest maturity to be over-predicted at a cooler site and under-predicted at a warmer site (Arnold 1959, Wang 1960).

$T_{\text{base}}$ and $T_{\text{opt}}$ were consistent across cultivars but thermal time of EFI and FIHM were cultivar specific. Differences in thermal time in FIHM between cultivars were small and of little practical importance, but differences in thermal time in EFI were large. Overall, delay in harvest date for ‘Fiesta’ was mainly due to the high thermal time requirement during EFI, since thermal duration of FIHM was not different from ‘Greenbelt’. Differences in thermal time to developmental stages are probably due to different developmental rates specific to each cultivar (Nowbuth and Pearson 1998). Lack of fit in both EFI and FIHM thermal time models for ‘Marathon’ were mainly due to within-cultivar variation. Observations of field-grown ‘Marathon’ consistently showed that it matured unevenly in the Darling Downs (Jauncey, P. 1998, pers. comm.), making harvest dates difficult to predict.

Predicted values were close to the observed values for chronological duration of FIHM in independent farm data (Fig 7.5b), and were closer to the 1:1 line than for chronological duration of EFI (Fig. 7.4b), with those predicted by optimised temperature coefficients. This agrees with reports that duration from sowing to floral initiation may be more difficult to predict than the duration from floral initiation to harvest (Diputado and Nichols 1989, Wurr et al. 1991a). Predicted accuracy for EFI in Brookstead were not as good as fitted values in Gatton College, possibly because crops grown under commercial conditions may be subjected to varying degrees of stress. For instance, commercial crops may have been sown at various depths in slightly different soil types over a large area, and probably experienced variable irrigation and fertiliser regimes, resulting in non-uniform rates of floral initiation compared to the more uniform environment imposed at Gatton College.

The EFI and FIHM thermal time models in this study are robust as they predicted chronological duration of EFI and FIHM for independent data of commercial field-
grown broccoli (60 crops) in a different location over two growing seasons. Hence, they can be used with confidence by farmers and researchers. Since optimised temperature response coefficients were the same across the range of cultivars, it is reasonable to use these temperatures as first approximations of $T_{\text{base}}$ and $T_{\text{opt}}$ for EFI and FIHM in other broccoli cultivars. These optimised temperature coefficients were further validated against independent crops of different cultivars for duration of EHM in Chapter 8.

7.4.2 Photoperiod response

In this experiment, independent effects of temperature and photoperiod were quantified using analysis by DEVEL. Based on controlled environment studies, broccoli is regarded as a long-day plant, responding to long photoperiods (16 h) by earlier (approximately 7-14 days) floral initiation (Gauss and Taylor 1969b, Fujime et al. 1988). The minimum irradiance ($2 \text{ W m}^{-2}$, equivalent to an additional $0.06 \text{ MJ m}^{-2} \text{ day}^{-1}$ since lights were on daily for 8 h) in the present study exceeded 1.5 W m$^{-2}$ (Friend 1969) which was found to be sufficient to saturate the photoperiod response of oilseed brassica ($B. \text{ campestris}$ L. cv Ceres). Thus, there was sufficient irradiance to detect a photoperiod response. However, photoperiod sensitivity was very low and only resulted in the slight delay of one day to floral initiation in ‘Fiesta’ and ‘Marathon’ but there was no delay in ‘Greenbelt’. This response was not consistent across sowings and 1 day is of little practical significance over an average duration of 22 days EFI. In no case was there evidence of earlier flowering in the long day (16 h) treatments in the present study. A recent study in the UK also showed no evidence for photoperiod sensitivity in three commercial cauliflower cultivars (Thapa 1994, Hadley and Pearson 1998). Therefore, a photoperiod effect can be ignored for practical purposes in the environment of south-east Queensland. Lack of photoperiod effect on total leaf number (Table 7.1) also supports this view. The cultivars in this study may not display a strong photoperiod response in south-east Queensland but may do so in higher latitudes with shorter photoperiods (< 11 h) (Tarakanov 1998). Another possibility is that the irradiance used in controlled environment studies was high (86-95 J s$^{-1}$ m$^{-2}$) and the observed decrease in chronological time of EFI with increasing photoperiod may have been due to increasing energy levels rather than
photoperiod (Gauss and Taylor 1969b). Implications of this have been discussed in Chapter 2 (Section 2.6.1).

No response to photoperiod was detected for the duration of FIHM by the analysis performed with DEVEL, which is consistent with other work (Marshall and Thompson 1987a, 1987b).

7.4.3 Solar radiation response

Inclusion of solar radiation did not improve the precision of the EFI model. This agrees with the literature since there is no report of any solar radiation effect for the duration of EFI (Miller et al. 1985, Miller 1988, Wiebe 1990, Wurr et al. 1995, Mourao and Hadley 1998). Work using shade covers to vary solar radiation transmissions showed that under lower radiation intensities, broccoli plants have a compensating mechanism whereby leaf area increased to increase light interception, and efficiency of conversion of this intercepted radiation in dry matter also increased (Mourao and Hadley 1998). Hence, broccoli development during EFI may not be sensitive to solar radiation.

ETT, which incorporates thermal time and solar radiation, predicted FIHM of each cultivar among the sowing dates with a lower F value than either chronological time or solar radiation, as in other work (Wurr et al. 1991a, 1992, Grevsen 1998), but was very close to the F value for thermal time. The equation relating ETT to thermal time reveals that the sensitivity of parameter \( a \) to solar radiation was very low. ‘Marathon’ had a higher value of \( a \), and hence is slightly more sensitive to solar radiation than ‘Fiesta’ or ‘Greenbelt’. However, values of \( a \) (0.045, 0.039 and 0.354 for ‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) for the cultivars used in this study are much lower than values of \( a \) for temperate cultivars (1.1, 1.6, 1.6 and 3.0 for ‘Citation’, ‘Prima’, ‘Cruiser’ and ‘Corvet’ respectively) growing in the UK (Wurr et al. 1991a) and are closer to values (0.09, 0.27 and 0.47 for ‘Flora Blanca’, ‘March’ and ‘December/January’ respectively) reported for cauliflower (Scaife et al. 1987, Wurr and Fellows 1998). Lack of sensitivity to solar radiation in the present study may be due to latitude in this study, in which high solar radiation generally occurs (see Table
3.4 in Chapter 3). Crops in temperate climates of higher latitudes grow at lower temperatures and usually lower radiation (Wurr et al. 1996b). These crops may be influenced by assimilate supply (which depends on solar radiation) and thus inclusion of radiation may improve predictive equations in those environments (Birch et al. 1998b, Nowbuth and Pearson 1998). In this study, crops were grown in a warmer sub-tropical environment where temperatures were close to optimum and solar radiation did not influence development greatly and thus, thermal time models provided satisfactory predictions of EFI and FIHM.

Recent work on broccoli head growth in Aarslev, Denmark (latitude 55°18’ N) showed that inclusion of solar radiation did not improve the accuracy of thermal time models for practical purposes (Grevsen 1998). Hence, thermal time models can be effectively applied to broccoli growing areas in temperate zones at higher latitudes.

### 7.4.4 Leaf number

The linear relationship between total leaf number and temperature for the data in this study is consistent with the literature reports (Fontes et al. 1967, Gauss and Taylor 1969b, Wiebe 1975, Miller et al. 1985, Miller 1988). At lower temperatures, broccoli plants flower at a lower node, and thus have fewer leaves than when temperatures are higher. Total leaf number of ‘Fiesta’ appears to be less sensitive to temperature than it is in ‘Greenbelt’ and ‘Marathon’, and this agrees with reports that the relationship between mean temperature and total leaf number is cultivar dependent (Fujime 1983, Booij 1987). There was also a significant linear relationship between thermal time duration of EFI and total leaf number at floral initiation for ‘Greenbelt’ and ‘Marathon’, as in cauliflower (Salter 1969) but not in ‘Fiesta’, confirming that total leaf number of ‘Fiesta’ is not very responsive to temperature. These differences in response to temperature and thus thermal time suggest that rate of leaf initiation may be temperature dependent in some cultivars (e.g. ‘Greenbelt’ and ‘Marathon’), but not in others (e.g. ‘Fiesta’). Alternatively, there may be a complex interaction, in which leaf initiation rate at temperatures above a threshold temperature is modified by other environmental factors (e.g. solar radiation) in some cultivars as reported for maize (Birch et al. 1998b), cauliflower and other crops (Mourao and Hadley 1998, Nowbuth...

In this work, higher temperatures (mean temperatures close to 20 °C) tended to shorten chronological EFI and FIHM. There was no need to estimate the end of the juvenile phase, as in a vernalisation approach, which is imprecise and may add to the error (Pearson et al. 1994, Hadley and Pearson 1998). Higher rates of development at higher temperatures may be related to an increased total leaf number and carbohydrate supply for floral initiation and development (Fontes and Ozbun 1972, Olesen and Grevsen 1997).
7.5 Conclusions

EFI and FIHM thermal time models in this study have three parameters: (i) thermal time calculated from (ii) $T_{\text{base}}$ and (iii) $T_{\text{opt}}$ for duration of EFI and FIHM, respectively, since $T_{\text{max}}$ was not determined. $T_{\text{base}}$ and $T_{\text{opt}}$ during both EFI and FIHM for three broccoli cultivars in a sub-tropical environment were 0 and 20 °C, respectively and consistent across cultivars, but thermal time duration of both EFI and FIHM were cultivar specific. Photoperiod only accounted for an additional 6%, 2% and 4% of the variation during EFI for ‘Fiesta’, ‘Greenbelt’ and ‘Marathon’ respectively. Due to the very low photoperiod sensitivity, photoperiod effect can be ignored in south-east Queensland. Inclusion of solar radiation did not improve precision of the EFI thermal time model and only slightly improved the FIHM thermal time model for ‘Marathon’. Both thermal time models proved to be robust as they accurately predicted floral initiation and harvest maturity of five cultivars in a commercial farm over two years. The thermal time approach was a satisfactory approach for developing an algorithm to predict the chronological duration of EFI and FIHM. Chapter 8 describes a one-step emergence to harvest model, and considers the relative attributes of the various models.
Chapter 8
Comparison and validation of thermal time models from emergence to harvest maturity

8.1 Introduction

Some thermal time models for broccoli use the same $T_{\text{base}}$ from sowing to harvest (Chung 1981, Titley 1987, Marshall and Thompson 1987a, 1987b, Dufault 1997) while others use slightly different $T_{\text{base}}$ according to phenological interval (Diputado and Nichols 1989). Specific models have also been developed to predict floral initiation (Chapter 7, Wurr et al. 1995, Tan et al. 1998) and harvest maturity from floral initiation (Chapter 7, Pearson and Hadley 1988, Wurr et al. 1991a, 1992). When the $T_{\text{base}}$ and $T_{\text{opt}}$ are similar in each phenological interval (Chapter 7), it may be possible to combine models predicting floral initiation and harvest maturity or use a single thermal time model to predict harvest maturity from emergence.

The objective of this study was to compare the predictive accuracy of thermal time models of three broccoli cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) from emergence to harvest maturity (EHM) (Model 1), from emergence to floral initiation (EFI) (Model 2), and from floral initiation to harvest maturity (FIHM) (Model 3), using RMSD and regression. Comparisons were made between a combined EFI and FIHM model (Model 4) and a single EHM model (Model 1). The thermal time models were tested on five cultivars (‘Fiesta’, ‘Greenbelt’, ‘Marathon’, ‘CMS Liberty’ and ‘Triathlon’) grown commercially over two years. In addition, the EHM model (Model 1) was further tested over five years using commercially grown crops in which floral initiation data were not available. The 1979-80 sowings from Titley (1985) were used for fitting $T_{\text{base}}$, $T_{\text{opt}}$ and calculating thermal time duration for EHM (Model 1) for three cultivars (‘Premium Crop’, ‘Selection 160’ and ‘Selection 165A’) and Model 1 was further validated using independent data from Titley’s 1983-84 sowings.
8.2 Materials and methods

8.2.1 Field experiment with photoperiod extension

A field experiment with photoperiod extension was conducted at the University of Queensland, Gatton College. Full details of the experimental design are presented in Chapter 3 (Section 3.1).

8.2.2 Commercial farm crops for testing the models

Crop ontogeny data to test the models were obtained from the commercial farm at the Brookstead location (cultural practices and location described in Chapter 3, Section 3.2) for 60 sowings of five cultivars (‘Fiesta’, ‘Greenbelt’, ‘Marathon’, ‘CMS Liberty’ and ‘Triathlon’) over two growing seasons (1997 and 1998). Details of the sowing dates are presented in Chapter 7 (Section 7.2.2). Further testing of the EHM thermal time model was conducted on additional data from 76 sowings in 1994, 1995 and 1996 (in addition to the 1997 and 1998 sowings). Since emergence and floral initiation data were not available for these sowings, emergence was assumed to be five days after sowing, which was the average duration for crops grown in 1997 and 1998, and the EFI and FIHM thermal time models could not be tested. ‘Fiesta’ (19 sowings) was sown on 4, 13, 23, 28 February, 4, 9, 14, 22, 29 March, 4, 11, 15, 22 April, 13, 20, 30 May, 3, 10, and 12 June 1996. ‘Greenbelt’ (25 sowings) was sown on 27 January, 7, 11, 17, 28 February, 16, 21, 25, 31 March 1994, 13, 18, 24, February, 6, 10, 16, 21, 25, 30 March 1995, and 18, 25, 29 February, 6, 11, 18, 27 March 1996. ‘Marathon’ (32 sowings) was sown on 4, 8, 14, 19, 25 April, 1, 8, 17, 25 May, 3, 12, 20, 28 June 1994, 4, 10, 16, 21, 27 April, 12, 25, 31 May, 7, 15, 23 June 1995 and 16 March, 1, 13, 19 April, 17, 28 May, 1, 5 June 1996.

8.2.3 Farm records
In addition to crop records for ‘Fiesta’ from the 1996 to 1998 seasons (3 years), and for ‘Greenbelt’ and ‘Marathon’ from the 1994 to 1997 seasons (4 years) mentioned in Section 8.2.2, crop schedules for sowing and harvest dates for ‘Greenbelt’ and ‘Marathon’ were also obtained for 1991 and 1993 from the same farm.

8.2.4 Titley’s experiments

To confirm the robustness of the single EHM thermal time model in the present study, it was further tested by re-analysing data of Titley (1985) who helped establish the broccoli industry in the Lockyer Valley in the 1980s (Titley 1987). Briefly, three cultivars, ‘Premium Crop’ (Arthur Yates & Co., Australia), ‘Selection 160’ and ‘Selection 165A’ (Henderson Seeds, Australia), were sown on 19 sowing dates in Gatton College at approximately 20 day intervals from March 1979 to March 1980 (1979-80 sowings) using a randomised complete block design with four replicates. The same cultivars (11, 19 and 6 sowings of ‘Premium Crop’, ‘Selection 160’ and ‘Selection 165A’, respectively) were grown on a commercial basis for export to south-east Asia in 1983 and 1984 (1983-84 sowings). Three rows, 0.5 m apart were sown for each cultivar on raised beds and plants were thinned to 0.3 m plant spacing (6-7 plants m⁻²) in all sowings. Irrigation and nutrients were supplied at rates to ensure non-limiting conditions were maintained and pests were controlled as required. The 1979-80 sowings were used for fitting \( T_{base} \) and \( T_{opt} \) using DEVEL (Holzworth and Hammer 1992), and calculating thermal time duration for EHM (Model 1) for the three cultivars and the 1983-84 sowings were used as independent data to validate Model 1. Since emergence and floral initiation data were not available for these sowings, emergence was assumed to be five days after sowing, and the EFI and FIHM thermal time models could not be tested.

8.2.5 Data analysis
In addition to the detailed descriptions given in Chapters 3 (Section 3.4) and 7 (Section 7.2.4), the following analyses were specific to this chapter.

Analysis of variance (ANOVA) was completed for thermal time duration of EHM in the field experiment with photoperiod extension to test the independent and interactive effects of photoperiod extension, sowing date and cultivar, using the general linear model (GLM) procedure of SAS.

The optimisation program, DEVEL (Holzworth and Hammer 1992), was used to determine the temperature and photoperiod responses of each cultivar from the experimental data for duration of EHM. Full details of the functions and use of DEVEL are presented in Chapter 7 (Section 7.2.4).

The EFI, FIHM and EHM thermal time models in this study have three parameters: (i) thermal time calculated from (ii) $T_{\text{base}}$ and (iii) $T_{\text{opt}}$ for duration of EFI, FIHM and EHM, respectively (Chapter 7). Thermal time ($^\circ\text{C d}$) for all models were calculated using the optimised $T_{\text{base}}$ and $T_{\text{opt}}$ of 0 and 20 $^\circ\text{C}$, respectively, as derived in Chapter 7 (Section 7.3.2). Root mean square deviation (RMSD), equivalent to the prediction error (Mikkelsen 1981, Yan and Wallace 1998), was used to compare models.

### 8.3 Results

#### 8.3.1 Time from sowing to harvest maturity

Chronological time (days) from sowing to maturity was plotted against sowing date for each of the three cultivars (Fig. 1.2 in Chapter 1) using the production schedules of sowing and harvest dates for seven years (1991, 1993 to 1998) maintained by the farm manager. A quadratic model (see Equation 2.6 in Chapter 2, Section 2.7.8) accounted for 76%, 49% and 12% of the variance of the chronological duration from sowing to harvest maturity for ‘Fiesta’ (Fig. 1.2a in Chapter 1), ‘Greenbelt’ (Fig. 1.2b in Chapter 1) and ‘Marathon’ (Fig. 1.2c in Chapter 1), respectively. Cold winters in 1994 and 1995 delayed maturity substantially whereas in a warmer year, such as
1993, maturity was advanced for ‘Greenbelt’ and ‘Marathon’. These quadratic models for EHM based on chronological time can be used to plan a crop sowing schedule but lack accuracy for predicting harvest dates.

### 8.3.2 Photoperiod, cultivar and sowing date effects

Analysis of variance for the EFI and FIHM intervals in the field experiment with photoperiod extension were presented in Chapter 7 (Section 7.3.1). Effects of photoperiod extension, cultivar, sowing date and their interactions on chronological and thermal time (°C d) duration of EHM were analysed by analysis of variance (Table 8.1). Photoperiod effect was not significant (P>0.05). The most notable interaction was between cultivar and sowing date. Later sowings significantly (P<0.01) increased the chronological duration of EHM, more so with ‘Fiesta’ than ‘Greenbelt’ and ‘Marathon’ (Fig. 8.1). This temporal trend is similar to the quadratic models in Fig. 1.2 (Section 1.2 in Chapter 1). Thermal time duration of for ‘Fiesta’, ‘Greenbelt’ and ‘Marathon’ were 1334, 1272 and 1305 °C d, respectively (Table 8.2), and was the sum of thermal time duration of EFI and FIHM for each cultivar.

**Table 8.1. Main and interactive effects of photoperiod extension (PP), sowing date (SD) and cultivar (CV) on the chronological time (days) and thermal time (°C d) duration of the interval from emergence to harvest maturity in broccoli [**, *, n.s. for P<0.01, P<0.05, not significantly different (P=0.05) respectively]. Dash (-) indicates no l.s.d. was calculated as the F-test was not significant at P=0.05.**

<table>
<thead>
<tr>
<th>Effect</th>
<th>PP</th>
<th>SD</th>
<th>CV</th>
<th>PP X SD</th>
<th>PP X CV</th>
<th>SD X CV</th>
<th>PP X SD X CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronological time</td>
<td>n.s.</td>
<td>**</td>
<td>**</td>
<td>n.s.</td>
<td>n.s.</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>l.s.d. (P=0.05)</td>
<td>-</td>
<td>2.19</td>
<td>0.71</td>
<td>-</td>
<td>-</td>
<td>2.74</td>
<td>2.84</td>
</tr>
<tr>
<td>Thermal time</td>
<td>n.s.</td>
<td>**</td>
<td>**</td>
<td>n.s.</td>
<td>n.s.</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>l.s.d. (P=0.05)</td>
<td>-</td>
<td>28.49</td>
<td>9.46</td>
<td>-</td>
<td>-</td>
<td>35.90</td>
<td>37.85</td>
</tr>
</tbody>
</table>
Fig. 8.1. Effect of sowing date [Julian day (Jday) where 1 = 1 Jan] and cultivar ['Fiesta' (●), ‘Greenbelt’ ( ■) and ‘Marathon’ (•)] on chronological duration (days) for eight sowing dates in a field experiment at Gatton College. The data presented are sowing date by cultivar interaction means (n = 6). Vertical lines indicate l.s.d. values at P=0.05 (for comparisons between sowing dates) for sowing date by cultivar interactions.
Table 8.2. Duration (mean ± s.e.) from emergence to floral initiation (EFI), from floral initiation to harvest maturity (FIHM) and from emergence to harvest maturity (EHM) for three broccoli cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) expressed as thermal time (°C d) grown under a range of photoperiod and temperature regimes in a field experiment at Gatton College, south-east Queensland, as estimated from optimisation routines using DEVEL. Thermal time was calculated from base and optimum temperatures of 0 and 20 °C. Means given were of 48 experimental units.

<table>
<thead>
<tr>
<th>Thermal time duration (°C d)</th>
<th>Cultivar</th>
<th>‘Fiesta’</th>
<th>‘Greenbelt’</th>
<th>‘Marathon’</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFI</td>
<td>670 ± 3.36</td>
<td>612 ± 5.43</td>
<td>627 ± 5.90</td>
<td></td>
</tr>
<tr>
<td>FIHM</td>
<td>664 ± 5.50</td>
<td>660 ± 5.05</td>
<td>678 ± 6.96</td>
<td></td>
</tr>
<tr>
<td>EHM</td>
<td>1334 ± 5.95</td>
<td>1272 ± 6.78</td>
<td>1305 ± 9.61</td>
<td></td>
</tr>
</tbody>
</table>

8.3.3 Accuracy of fitted values from optimised $T_{base}$ and $T_{opt}$

Chronological duration of EHM (Model 1) was fitted from the optimised parameters provided by DEVEL (Fig. 8.2a) and compared to Model 4 (the combination of EFI and FIHM models, Fig. 8.3a). The goodness of fit of the fitted values for the three cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) for the two models were similar, except Model 4 was closer to the 1:1 line, although there was still a slight tendency towards over prediction.
Fig. 8.2. (a) Comparison between fitted and observed duration (days) from emergence to harvest maturity for three broccoli cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) when grown at Gatton College; and (b) comparison between predicted and observed duration (days) from emergence to harvest maturity for independent data from five broccoli cultivars (‘Fiesta’, ‘Greenbelt’, ‘Marathon’, ‘CMS Liberty’, and ‘Triathlon’) grown on a commercial farm in Brookstead in 1997 and 1998, using a single emergence to harvest maturity model (Model 1). Predicted duration was calculated using $T_{\text{base}}$ and $T_{\text{opt}}$ of 0 and 20 °C, respectively, derived using the optimisation routine, DEVEL, from a field experiment at Gatton College, south-east Queensland.
Fig. 8.3. (a) Comparison between fitted and observed duration (days) from emergence to harvest maturity for three broccoli cultivars (‘Fiesta’, ‘Greenbelt’ and ‘Marathon’) grown at Gatton College; and (b) comparison between predicted and observed duration (days) from emergence to harvest maturity for independent data from five broccoli cultivars (‘Fiesta’, ‘Greenbelt’, ‘Marathon’, ‘CMS Liberty’, and ‘Triathlon’) grown on a commercial farm in Brookstead in 1997 and 1998, using a combined emergence to floral initiation and floral initiation to harvest maturity model (Model 4). Predicted duration was calculated using base and optimum temperatures of 0 and 20 °C, respectively, derived using the optimisation routine, DEVEL, from a field experiment at Gatton College, south-east Queensland.
8.3.4 Evaluation of models against independent farm data

Model 1 (Fig 8.2b) and Model 4 (Fig. 8.3b) developed from the experimental data at Gatton College were applied to the independent farm data at Brookstead. The coefficient of determination, slope of the line and intercept for Model 4 were better than those for Model 1. This improvement was probably due to the breakdown into components of the distinct phenological intervals which improved the precision of predictions for individual sowings. The RMSD of Model 4 (3.3) was similar to that in Model 1 (3.0). Although Model 4 best predicted the chronological duration of EHM, Model 1 can be used instead of Model 4 in cases where data on floral initiation are not available.

Since floral initiation data were not available from 1994 to 1996, additional DEVEL analysis could only be conducted for the duration of EHM. Model 1 predicted 73% of the variation for the pooled analysis with 136 sowings of five cultivars (‘Fiesta’, ‘Greenbelt’, ‘Marathon’, ‘CMS Liberty’ and ‘Triathlon’) over five years (1994 to 1998) (Fig. 8.4). The slope of the line and the intercept in Fig. 8.4 were similar to those in Fig. 8.2b, showing that the model can be applied reliably over an extensive data set (Fig. 8.4).

When floral initiation data were available, Model 3 (FIHM) (Fig. 7.5b in Chapter 7, Section 7.3.7) predicted harvest maturity with greater precision than Model 1 (EHM) (Fig. 8.2b). More predicted values in Model 3 fall closer to the 1:1 line than in Model 1 although the RMSD of both models were similar. Precision was improved since the variation which occurred during EFI was removed.
Observed duration (d) of EHM

Predicted duration (d) of EHM

$y = 0.73x + 29.08 \quad (r^2 = 0.73, P<0.01), \quad \text{RMSD} = 4.36, \quad n = 136$

Fig. 8.4. Comparison between predicted and observed duration (days) from emergence to harvest maturity for independent data from five broccoli cultivars (‘Fiesta’, ‘Greenbelt’, ‘Marathon’, ‘CMS Liberty’, and ‘Triathlon’) grown on a commercial farm in Brookstead in 1994, 1995, 1996, 1997 and 1998, using a single emergence to harvest maturity model. Predicted duration was calculated using $T_{\text{base}}$ and $T_{\text{opt}}$ of 0 and 20 °C, respectively, derived using the optimisation routine, DEVEL, from a field experiment at Gatton College, south-east Queensland.

8.3.5 Evaluation of the EHM model against Titley’s data

$T_{\text{base}}$ and $T_{\text{opt}}$ of 0 and 20 °C derived using DEVEL, were found for all three cultivars in the 1979-80 sowings, these temperatures falling within the 10% confidence interval for individual cultivars for duration of EHM. Thermal time duration of EHM, (calculated using $T_{\text{base}}$ and $T_{\text{opt}}$ of 0 and 20 °C) for ‘Premium Crop’, ‘Selection 160’ and ‘Selection 167A were 1355 ± 17, 1305 ± 22, and 1486 ± 25 °C d (n = 19), respectively. Chronological duration of EHM was fitted from the optimised temperature parameters by DEVEL (Fig. 8.5a) and the regression shows a good fit.
The model developed from the 1979-80 sowings was then applied to independent data from the 1983-84 sowings. The coefficient of determination for predicted values using optimised temperature coefficients accounted for 91% of the observed data for chronological duration of EHM in independent commercial crop data (Fig. 8.5b), in the pooled analysis with three cultivars (‘Premium Crop’, ‘Selection 160’, ‘Selection 165A’). Most of the points fall close to the 1:1 line and RMSD for the pooled data was 2.5 days.
Fig. 8.5. (a) Comparison between fitted and observed duration (days) from emergence to harvest maturity for three broccoli cultivars (‘Premium Crop’, ‘Selection 160’ and ‘Selection 165A’) when sown during 1979-80, and (b) comparison between predicted and observed duration (days) from emergence to harvest maturity for independent data from three broccoli cultivars (‘Premium Crop’, ‘Selection 160’ and ‘Selection 165A’) sown commercially during 1983-84 at Gatton College, using a single emergence to harvest maturity model. Predicted duration was calculated using $T_{\text{base}}$ and $T_{\text{opt}}$ of 0 and 20 °C, respectively, derived using the optimisation routine, DEVEL, from the 1979-80 sowings at Gatton College, south-east Queensland.
8.4 Discussion

Traditionally, curvilinear relationships, like those in Fig. 1.2 (Section 1.2 in Chapter 1), have been used to plan a sowing and marketing schedule but variations in prevailing weather conditions can cause deviations from the plan. Incidentally, the convex curves of Fig. 1.2 become concave curves in the Northern Hemisphere where progressive sowings experience warmer rather than cooler environments (Wurr et al. 1991a, Dufault 1997). This chapter describes and compares four models of broccoli development, two from emergence to harvest (Models 1 & 4), one from emergence to floral initiation (Model 2) and one from floral initiation to harvest maturity (Model 3). Each can be used to better manage the interactions between crop sowing schedules, cultural practices and marketing. Details of Models 2 and 3 are described in Chapter 7 and applications of the four models are discussed in Chapter 9.

When floral initiation data (Chapter 4) were available, precision in predicting harvest maturity were improved [RMSD of 1.9 (Gatton College), 2.9 (Brookstead) days] by using the FIHM model (Model 3). Although the FIHM model (Model 3) can be applied independently of the EFI model (Model 2), prediction of floral initiation using the EFI model is useful, since sowing dates can be adjusted to avoid frost and high temperature periods at critical stages (e.g. floral initiation) of crop development (Bjorkman and Pearson 1998, Chapter 5).

The single EHM model (Model 1, RMSD of 3.0 days) could predict harvest maturity almost as well as the combined EFI and FIHM models (Model 4, RMSD of 3.3 days) since the same optimised temperature coefficients of 0 and 20 °C were used, although predictions by Model 4 were closer to the 1:1 line than for Model 1. Thus, the single EHM model approach, which was also used by other researchers, (Chung 1981, Titley 1987, Marshall and Thompson 1987a, 1987b, Dufault 1997) can be used by farmers and researchers to predict harvest dates from emergence, and plan sowing schedules. A sowing schedule might be planned using mean weekly temperatures, but by progressively applying the model with daily temperatures, deviation between the actual and planned predicted harvest date will become apparent. The single EHM model (Model 1) in this study is more robust than a site specific model (Chung 1981).
and it adequately predicted chronological duration of EHM (RMSD of 4.4 days) for five cultivars of commercial field-grown broccoli over five growing seasons. This compares favourably with the Scottish model which predicted the duration from sowing to harvest maturity within ± 7 days for nine out of ten crops (Marshall and Thompson 1987a). Fitted values from the 1979-80 sowings at Gatton College (Titley 1985) confirmed that $T_{\text{base}}$ and $T_{\text{opt}}$ were 0 and 20 °C, respectively for additional cultivars and thermal time of EHM was cultivar specific. This single EHM model (Model 1) was further validated by independent data from the 1983-84 sowings, showing that the EHM model is quite robust, as it could be applied to different cultivars grown more than ten years earlier. The simple thermal time, modified thermal time, and non-linear rectangular hyperbola models described in Chapter 2 (Section 2.7.2) only accounted for 23%, 64% and 85% of the variation, respectively when tested on the same independent data from the 1983-84 sowings (Titley 1985, 1987). Model 1 in this study accounted for 91% of the variation and hence, is an improvement on the Gatton models (Titley 1985, 1987). This improvement was possible due to selection of cardinal temperatures that were physiologically sound, using DEVEL. Based on these data, it is reasonable to apply $T_{\text{base}}$ and $T_{\text{opt}}$ of 0 and 20 °C, respectively to the EHM interval for other broccoli cultivars.

### 8.5 Conclusions

Since the same optimised coefficients of 0 and 20 °C were used in all models, a single emergence to harvest maturity model (Model 1) can predict harvest maturity almost as well as a combined EFI and FIHM model (Model 4). Similarly, rigorous testing on independent data obtained for three cultivars (‘Premium Crop’, ‘Selection 160’ and ‘Selection 165A’) grown more than a decade earlier, showed that Model 1 predicted the duration of EHM well. The FIHM model (Model 3) provided the best prediction for harvest maturity. Prediction of floral initiation using the EFI model (Model 2) is useful for heat and frost avoidance during floral initiation.
Chapter 9

General Discussion

This study has produced models to assist broccoli farmers with crop scheduling and cultivar selection in south-east Queensland. A reliable and repeatable method to detect floral initiation was developed as a basis for subsequent work. Thermal time predictive models were developed to improve production and marketing arrangements which have to be made in advance of crop harvest. The interaction between cultivar and environment was described to enable farmers to sow the best adapted cultivar for each sowing date, and optimise yield and quality. However, there may be some inaccuracy in prediction using these models when plants are grown under extreme conditions such as severe frosts. Sub-zero temperatures can retard growth and reduce yield and quality. Conditional statements for frost risk are needed in developing thermal time models. This chapter discusses the results in relation to the objectives listed in Chapter 1 (Section 1.5), provides linkages between the experiments, places the work in context, and points to future work arising from this study. The relationship between the chapters of this thesis is depicted in Fig. 9.1.

Fig. 9.1. Relational diagram of the work reported in this thesis. Broken lines show the flow of information.

9.1 Detection of floral initiation
When developing crop models for prediction of ontogeny, detection of floral initiation is essential (Chapter 4). Since there was limited information in the literature defining floral initiation, a set of electron micrograph standards of the apical meristem was made for the transition from the vegetative to the advanced reproductive stage (Tan et al. 1998). Floral initiation was determined when a new reproductive structure (first-order floral branch primordia) first developed on the shoot apex. This method was reliable and repeatable and was consistent with the approach used by Salter (1969) and Moncur (1981) for the closely related cauliflower. The morphological development of broccoli and cauliflower apices during the transition from vegetative to reproductive stages is similar, but quite different from other brassicas such as radish and oilseed brassica (Moncur 1981, Smith and Scarisbrick 1990).

The present study resolves an area of discrepancy in the definition of the apex diameter at floral initiation. Mean apex diameter at floral initiation was $500 \pm 3 \mu m$ in this study, which is consistent with the findings of Wurr et al. (1995). In contrast, apex diameter during the transitional and reproductive stages was reported to be greater than $2000 \mu m$ by Fyffe and Titley (1989). However, Fyffe and Titley (1989) used low magnification ($\times20$) to view the apex, and this could have reduced the accuracy of the measurements. Other workers (e.g. Wurr et al. 1995) used higher magnifications ($\times100 \text{ or } \times150$).

### 9.2 Yield and quality

Knowledge of genotype and environment interactions will assist farmers in matching cultivars to time of sowing and hence optimise yield and quality. FWHI increased as sowings progressed from early to late autumn (Chapter 6). As GSM minimum temperature decreased, fresh weight of tops decreased linearly while fresh weight harvest index increased linearly. There was no definite relationship between fresh weight of tops or fresh weight harvest index and GSM minimum temperatures $\geq 10 ^{\circ}C$. Retarded vegetative growth and increased partitioning into the reproductive head, may be the result of progressively lower GSM minimum temperatures during winter (Gauss and Taylor 1969b, Kage and Stutzel 1999). Quality attributes of head shape,
branching angle, bud size and evenness, and bractiness were determined by cultivars rather than environmental factors (Chapter 6). A broken linear relationship was fitted between both bud colour and cluster separation and GSM minimum temperature for the cultivar ‘Marathon’ in this study. Broken linear models are more biologically meaningful than third order polynomial equations used by Dufault (1996). Principal components analysis confirmed that overall quality of broccoli was affected more by genotype than by environment in the present study.

Broccoli yield and quality were not affected by photoperiod in this study (Chapter 6). There was no evidence to justify speculations that broccoli yield and quality may be affected by photoperiod (Chung 1985a, Chung and Strickland 1986, Thompson and Taylor 1970) in the sub-tropical environment of south-east Queensland.

Adverse growing conditions such as severe frosts can result in loss of head yield and quality. The vegetative and floral initiation stages were more sensitive to sub-zero temperatures than the buttoning stage (Chapter 5). The floral initiation stage appeared to be the most sensitive to sub-zero temperature injury, as yields were reduced at $-1 \, ^\circ C$ and $-3 \, ^\circ C$, and the shoot apices were killed at $-5 \, ^\circ C$, resulting in no yield. There was no yield reduction at the buttoning stage for treatments at $-1 \, ^\circ C$ and $-3 \, ^\circ C$ and shoot apices survived $-5 \, ^\circ C$ but produced poor quality heads. The uneven bud size and poor cluster separation in these heads may have resulted from localised developmental arrest (Bjorkman and Pearson 1998) which occur during floral bud development and internode elongation (Carr and Irish 1997). Although this phenomenon was reported for broccoli subjected to heat stress ($> 35 \, ^\circ C$), similar symptoms of uneven bud size in this study suggest that it also occurs with sub-zero temperature stress.

### 9.3 Thermal time models
EFI and FIHM thermal time models in this study have three parameters: (i) thermal time calculated from (ii) $T_{\text{base}}$ and (iii) $T_{\text{opt}}$ for the duration of EFI and FIHM, respectively (Chapter 7). The $T_{\text{base}}$ and $T_{\text{opt}}$ of 0 and 20 °C for the duration of EFI determined from the present study, are consistent with a study in New Zealand (Diputado and Nichols 1989) where $T_{\text{base}}$ and $T_{\text{opt}}$ of 1 and 21 °C respectively, were calculated using the least coefficient of variation method. The $T_{\text{base}}$ and $T_{\text{opt}}$ of 0 and 20 °C for the duration of FIHM is generally consistent with most literature sources. The optimised $T_{\text{base}}$ of 0 °C also agrees with other results (Marshall and Thompson 1987a, Wurr et al. 1991a, 1992, Grevsen 1998), but is lower than the report of 3 °C by Diputado and Nichols 1989. The $T_{\text{opt}}$ of 20 °C in this study is slightly higher than the values of 15 and 17 °C reported for temperate broccoli cultivars growing in the UK (Wurr et al. 1991a) and Denmark (Grevsen 1998), respectively. $T_{\text{base}}$ and $T_{\text{opt}}$ were consistent across cultivars but thermal time of EFI, FIHM and EHM were cultivar specific. Both EFI and FIHM thermal time models in this study are robust, as they predicted chronological duration of EFI and FIHM for independent data of commercial field-grown broccoli over two growing seasons.

Since the optimised temperature coefficients were the same for the different phenological intervals, the single EHM approach (RMSD of 3.0 days) could be used (Chapter 8). This EHM model was robust and it adequately predicted chronological duration of EHM for independent data of five cultivars of commercial field-grown crops on the Darling Downs over five growing seasons. This model may be more accurate than the Scottish model which predicted the duration from sowing to harvest maturity within ± 7 days for nine out of ten crops in Scotland (Marshall and Thompson 1987a). The single EHM model was also generated from 1979-80 sowings of three cultivars from Titley (1985). When this single EHM model was subsequently tested on independent data from 1983-84 sowings, it also predicted harvest maturity well (Chapter 8). The single EHM model developed in this study accounted for 91% of the variation in chronological duration of EHM. In the three Gatton models developed by Titley (1985, 1987), which were based on simple thermal time, modified thermal time, and non-linear rectangular hyperbola (Chapter 2, Section 2.7.2), only 23%, 64% and 85% of the variation in chronological duration was
accounted for when tested using the same independent data from the 1983-84 sowings.

The thermal time models developed in Chapters 7 and 8 may not produce accurate predictions if broccoli plants are subjected to adverse conditions such as a severe frost (Chapter 5). Pot-grown plants treated during floral initiation at $-1 \, ^\circ C$ and $-3 \, ^\circ C$, and during the buttoning stage at $-5 \, ^\circ C$ produced heads that were significantly smaller and with lower weight. Leaves of pot-grown plants were damaged at $-5 \, ^\circ C$ (Chapter 5) and plant growth appeared to be retarded after the treatment, possibly due to impairment of chloroplast functioning. Photosynthesis would be substantially reduced after freezing due to disturbance of both chloroplastic processes and stomatal opening (Bauer et al. 1992, 1994). This suggests that crop development models based only on simple thermal time without restrictions may not apply after severe frosts have caused freezing injury, since a ‘shock’ or ‘stress’ over-rides normal developmental processes. Conditional statements of the following are needed in thermal time models for crops likely to be severely affected by frost, resulting in reduced developmental rates, yield and quality:

$$\text{If } T_{\text{min}} < x \, ^\circ C, \text{ risk of reduced developmental rate, yield & quality is increased,}$$

where $x =$ sub-zero temperatures ($^\circ C$).

Consequently, production, management and marketing schedules may be altered to take into account frost risk.

Photoperiod sensitivity during EFI, as calculated using DEVEL (Holzworth and Hammer 1992) was very low (Chapter 7). The lack of photoperiod effect on total leaf number supports the analyses of temperature and photoperiod effects, since significant photoperiod sensitivity would result in a change in total leaf number on each sowing date. Results in this study are similar to those from UK research which showed no evidence for photoperiod sensitivity in three commercial cauliflower cultivars (‘Plana’, ‘Kathmandu Local’ and ‘Snowball-16’) growing under different photoperiods ($9, 12, 15, 18 \, \text{h day}^{-1}$). Photoperiod also had no effect on final leaf number in cauliflower cultivars. (Thapa 1994, Hadley and Pearson 1998). No
response to photoperiod was detected for the duration of FIHM, which agrees with other work (Marshall and Thompson 1987a, 1987b). The cultivars used in the present study did not display a strong photoperiod response in south-east Queensland but may do so in higher latitudes with photoperiods < 11 h (Tarakanov 1998). Hence, the photoperiod response of these cultivars should be studied further in temperate latitudes.

Inclusion of solar radiation did not improve the precision of the EFI model, which is consistent with the literature (Miller et al. 1985, Miller 1988, Wurr et al. 1995, Mourao and Hadley 1998). ETT, which incorporates thermal time and solar radiation, predicted FIHM of each cultivar among the sowing dates with a lower F value than either chronological time or solar radiation, which also agrees with other research (Wurr et al. 1991a, 1992, Grevesen 1998, Klaring 1998), but was very close to the F value for thermal time (Chapter 7). To this extent, the environment in south-east Queensland differs substantially from most other broccoli-producing areas in higher latitudes, which usually have lower irradiance (Wurr et al. 1996b) and a wider range of photoperiods. Recent work in Aarslev, Denmark (latitude 55°18’ N) showed that inclusion of solar radiation did not give any practical improvement in thermal models for head growth (Grevesen 1998). These results suggest that thermal time models without inclusion of solar radiation, can be effectively applied to locations in higher latitudes.

### 9.4 Applications of thermal time models

The single EHM model (RMSD of 3.0 days) could predict harvest maturity almost as well as the combined EFI and FIHM models (RMSD of 3.3 days) since thermal time was calculated using the same $T_{\text{base}}$ and $T_{\text{opt}}$ of 0 and 20 °C, respectively (Chapter 8). A single EHM model approach, which was also used by other researchers (Chung 1981, Titey 1987, Marshall and Thompson 1987a, 1987b, Dufault 1997), can be used by growers and researchers to predict harvest dates from emergence, and plan sowing schedules. A sowing schedule might be planned using mean weekly temperatures, but by progressively applying the model with daily temperatures, deviation between the actual and planned predicted harvest date will be apparent.
When floral initiation data (Chapter 4) are available, precision in predicting harvest maturity can be improved [RMSD of 1.9 days (Gatton College), 2.9 days (Brookstead)] by using the FIHM model, since variation which occurs during EFI is removed. The FIHM model in this study is precise and gives predictions of comparable accuracy to the more complex maturity models developed at Wellesbourne, UK and Denmark, which reported RMSD of 1.73 – 3.26 days (Wurr 1992) and 4 – 5 days (Grevsen 1998), respectively. The FIHM model in this study only requires maximum and minimum daily temperatures, thermal time requirement and floral initiation data. It does not need solar radiation and sensitivity of individual cultivars to solar radiation used in the Wellesbourne maturity models (Wurr et al. 1991a, 1992, Chapter 7). This may be because of the non-limiting radiation conditions in south-east Queensland, meaning predictive models do not need to take account of low radiation effects.

Although the FIHM model can be applied independently of the EFI model, the EFI model is useful for predicting floral initiation. With the development of advanced weather forecasting systems, it has become possible to predict the likely date of the first and last frost. The number and severity of frosts in north-eastern Australia can be predicted with increasing accuracy using the Southern Oscillation Index (SOI) (Stone et al. 1996a, 1996b). Broccoli plants are more sensitive to freezing injury (Chapter 5) and high temperatures (≥35 °C) (Bjorkman and Pearson 1998) during floral initiation. The thermal time models proposed in this study (Chapter 7), incorporating frost risk using conditional statements (see Section 9.3), can be combined with advanced weather forecasting to examine risks of crop exposure to high or low temperatures at critical stages of crop development (e.g. floral initiation). It will then be possible to adjust sowing dates or management options to minimise frost or heat damage as part of a decision support system for broccoli production.

The thermal time models developed in this study may be adapted to simulate the potential impact of long term climatic trends such as increased temperatures associated with global warming on broccoli ontogeny (Wurr et al. 1996a). The
models can also be integrated into decision support systems for farmers. Hence, farmers will have a quantitative tool for decision making and marketing.

9.5 Cultivar selection

‘Fiesta’ was the best performing cultivar in this study, followed by ‘Greenbelt’ and ‘Marathon’. ‘Fiesta’ had significantly higher head shape and branching angle ratings than ‘Greenbelt’ and ‘Marathon’. Bud evenness ratings for ‘Fiesta’ and ‘Greenbelt’ were significantly higher than for ‘Marathon’. ‘Marathon’ was the inferior cultivar, as bud colour and cluster separation ratings only deemed heads suited for export when GSM minimum temperatures were < 8 °C. The EFI thermal time model explained 89%, 70% and 53% of the variation during EFI, and the FIHM thermal time model explained 90%, 80% and 36% of the variation during FIHM for ‘Fiesta’, ‘Greenbelt’ and ‘Marathon’, respectively. ‘Marathon’ had a consistently poor fit to the thermal time models mainly due to within-cultivar variation. ‘Marathon’ is not well adapted to environmental conditions in south-east Queensland, and its gradual replacement by ‘Triathlon’ and ‘Decathlon’ (Sakata, USA), which are superior versions of ‘Marathon’ bred by Sakata (Sakata, 1997), is fully justified.
9.6 Conclusions

In conclusion, this study has achieved the objectives listed in Chapter 1 (Section 1.5). A summary of how the objectives have been achieved follows.

1. **To provide reliable and repeatable method of detecting floral initiation in broccoli.** A set of scanning electron micrographs of shoot apices provided the basis for detecting floral initiation consistently in subsequent work.

2. **To describe and quantify the effect of sub-zero temperatures imposed at sequential developmental stages on leaf and shoot apex mortality, and head yield and quality.** Freezing injury can reduce broccoli yield and quality and retard plant growth. Plants at the vegetative and floral initiation stages were more sensitive to sub-zero temperatures than at the buttoning stage. Crop development models based only on simple thermal time without restrictions will not predict yield and maturity if broccoli crops are frost-damaged.

3. **To quantify the response of head yield and quality to temperature and photoperiod in 3 cultivars grown under field conditions in a sub-tropical environment.** ‘Fiesta’ was the best performing cultivar in this study, followed by ‘Greenbelt’ and ‘Marathon’ when assessed on the head quality attributes of head shape and branching angle. Yield and quality were not influenced by photoperiod. As GSM minimum temperatures decreased, fresh weight harvest index increased linearly. There was no definite relationship between fresh weight harvest index and GSM minimum temperatures $\geq 10 \, ^{\circ}\text{C}$. Head quality was predominantly determined by cultivar. Environmental influences contributed relatively little to quality attributes investigated in this study.

4. **To quantify the response of crop development to temperature and photoperiod in 3 cultivars from emergence to floral initiation, and from floral"
initiation to harvest maturity. Photoperiod sensitivity for broccoli development was very low. Thermal time models in this study were based on thermal time, calculated from optimised $T_{\text{base}}$ and $T_{\text{opt}}$ of 0 and 20 °C, respectively, which were consistent across cultivars. Thermal time requirements for each phenological interval were cultivar specific.

5. To compare and validate thermal time models from emergence to harvest maturity. The single EHM model was useful for predicting harvest maturity when floral initiation data were not available. The combination of the EFI and FIHM models provided the best prediction for duration of EHM. The FIHM model predicted harvest maturity with the best accuracy, since variation which occurred during the EFI interval was removed. Prediction of floral initiation using the EFI model is useful for timing cultural practices, and avoiding frost and high temperature periods.

Together, the data herein are a foundation for a decision support system to manage the sequence of sowings on commercial broccoli farms.
9.7 Suggested future work

Activities to further improve the generality of the models presented in this study, and to enhance their applications, are summarised below:

(i) Development of ‘user friendly’ software for pre-scheduling broccoli is now possible, using the thermal time, yield and quality, and frost risk models developed in this study. This software can be integrated into decision support systems for farmers.

(ii) More controlled freezing experiments are needed to determine plant tissue freeze-killing temperatures more accurately. A radiation frost chamber can better simulate natural frost conditions and observation of ice formation in plants can be improved using infrared video thermography. Further studies into cold acclimation and breeding broccoli with enhanced freeze-resistance would be beneficial.

(iii) Further testing of the models is required in diverse environments (e.g. in higher latitudes) under different temperature, photoperiod (< 11 h) and solar radiation conditions.

(iv) Since the models did not explain variability in development and quality very well for ‘Marathon’, other processes, such as genetic factors, may be operating. This warrants further studies to develop broccoli cultivars with improved adaptability to diverse environmental conditions and better genetic uniformity in developmental rates and quality.

(v) The work reported in this thesis did not measure vernalisation responses, rate of head growth after floral initiation, and dry matter production and partitioning in broccoli. Further work in these areas may lead to better understanding of broccoli physiology, thus facilitating the development of a dynamic simulation model for broccoli development.


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