THE EFFECT OF ENVIRONMENTAL FACTORS ON FLOWER COLOUR AND PETAL NUMBER OF *POTENTILLA FRUTICOSA* L.

by

Masumi Robertson

A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
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in
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ABSTRACT

Robertson, Masumi. M.Sc., The University of Manitoba, November, 1984. The Effect of Environmental Factors on Flower Colour and Petal number of *Potentilla fruticosa* L. Major Professor; Louis M. Lenz.

The study was conducted to determine the types of pigments present in the petals and environmental factors affecting the changes from cyanic to acyanic flower colour and in petal number within the taxon *Potentilla fruticosa*.

Chromatographic identification of petal pigments was conducted. Pigment concentrations were determined spectrophotometrically for plants grown under field conditions and in controlled environment. Petal number was recorded under the same conditions.

The 'red' colour of petals is due to the presence of anthocyanins (cyanic) and the change of flower colour is due to reduction of anthocyanin synthesis. Carotenoids were also present in yellow, orange and salmon flowers.

Linear regression analysis between various environmental factors and anthocyanin and carotenoid concentrations indicated that higher temperature resulted in a decrease in anthocyanin concentrations and the effect on carotenoid concentration varied among taxa. The temperature effect was more pronounced with higher temperature. There was no effect by moisture.

Petal number was affected by both temperature and moisture factors around the time of flower initiation. However there was no significant effect as the result of temperature treatment. Petal number change appears to be a result of the combined effect of environmental factors.

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Chapter I

INTRODUCTION

Shrubby cinquefoil, Potentilla fruticosa L., is a native dwarf shrub with yellow or white flowers. The plant has a long flowering period during the growing season, thus is desirable landscape material.

Breeding programs have been carried out in the northern hemisphere to develop other flower colours; red, orange or pink, and/or extra petals. The breeding and selection program at the University of Manitoba was initiated in 1968. It was observed that flower colour or petal number changed during the growing season.

This study was undertaken to determine the types of pigments present in the petals and the effects of environmental factors on flower colour and petal number of various taxa and University of Manitoba selection lines.

Chapter II

LITERATURE REVIEW

Potentilla fruticosa L. is a dwarf shrub of the family Rosaceae. The genus contains about 500 species of annual and perennial herbs and a few shrubs (Bailey and Bailey, 1976). The native forms, distributed circumpolar in the northern hemisphere, are yellow or white in flower colour with a petal number of five. In addition to native forms, there are many cultivars with colours ranging from white to dark yellow, some being cyanic (containing anthocyanins), or with a petal number of more than five.

2.1 FLOWER PIGMENTS

There are three major groups of plant pigments; chlorophylls, flavonoids, and carotenoids. Chlorophylls are responsible for the green colouration of leaves and stems (Holt, 1965). This group of pigments is involved in the plant photosynthetic functions (Katz, 1972; Whittingham, 1965).

The flavonoids are white, yellow, and shades of red to blue pigments (Swain, 1965). They are present in all parts of plants, especially apparent in areas where the pigments are not masked by the presence of chlorophylls. The flowers containing these pigments attract pollinating vectors such as birds and insects, thus subsequent seed set will ensure plant survival (Harborne, 1967 and 1972).

The carotenoids are the yellow to red pigments in flowers, fruits, leaves and other parts of plants (Goodwin, 1965). Their function includes photosynthesis, photoprotection, photoresponses, and reproduction through the attraction of pollinating vectors and aid seed dispersal (Burnett, 1965; Krisky, 1971).

2.1.1 Flavonoids

Flavonoids are water soluble pigments made up of several classes of compounds. The basic structure consists of two benzene rings joined together by a three carbon link. Various classes of these pigments are grouped according to the different oxidation state of the three carbon link (Swain, 1965).

There are three major classes and five or more minor classes of flavonoids, namely, anthocyanins, flavones and flavonols; chalcones, aurones, dihydrochalcones, flavonones, and dihydroflavonoles, respectively (Harborne and Mabry, 1982).

Colour expression of each class varies within a class, depending on substitution on the molecule, however generally;

anthocyanins	yellow, orange, red, violet, blue to black
flavones	white, ivory to cream
flavonols	white, ivory, cream, to yellow
chalcones	yellow
aurones	yellow to orange

Many flavonoids are colourless (Harborne, 1959, 1965, 1967, and 1972).

Flavonoids are synthesized through the general phenylpropanoid pathway and the flavonoid glycoside pathway (Ebel and Hahlbrock, 1982) (Figure 1). Phenylalanine produced through the shikimate pathway is a common precursor to many phenolic compounds. The first common intermediate of all flavonoids is a chalcone, produced from 4-coumaroyl CoA (ring B and a three carbon bridge) and three two carbon units (ring A). Flavonone is produced from the chalcone by an isomerase enzyme, then the middle ring is modified to produce other classes of flavonoids. Further modification by hydroxylation, methylation, glycosylation and acylation, probably occurs after the formation of the first intermediate in each class of flavonoids. Anthocyanins are some of the end products in the pathway and biosynthesis may be closely related to flavonol biosynthesis.

Flavonoids have a very wide distribution pattern. They are common constituents in the higher plant orders; bryophyta, ferns, gymnosperms and angiosperms, but are of restricted distribution in the lower orders; bacteria,

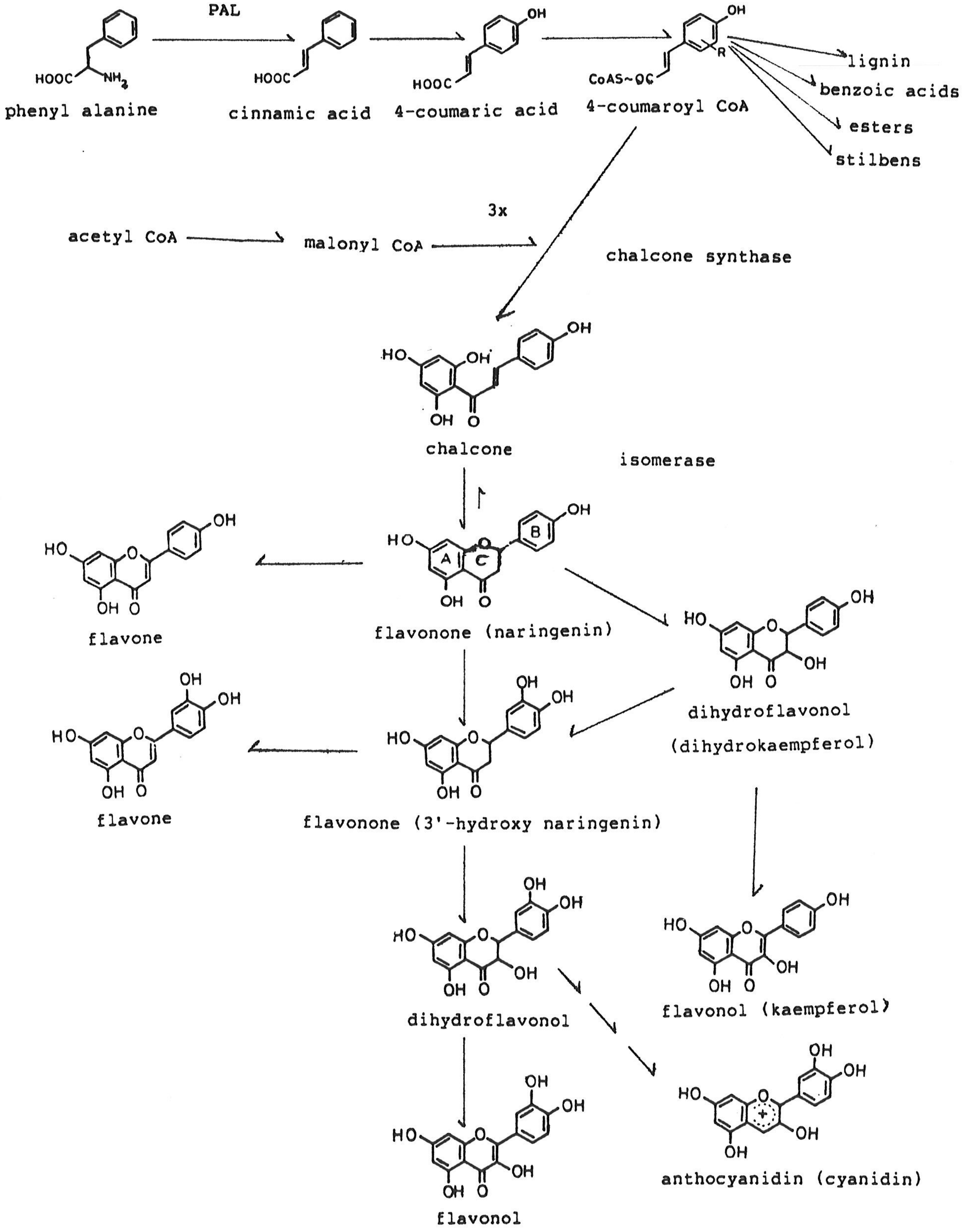

Figure 1: Biosynthesis of Flavonoids

algae, and fungi (Geissman, 1962; Harborne, 1967 and 1972; Harborne and Williams, 1982). Anthocyanins are characteristic of higher plants with particularly frequent occurrence in the angiosperms (Harborne, 1965). They occur more commonly as glycosides and there is a complete absence of naturally occurring anthocyanidins (aglycones) (Geissman, 1962; Harborne 1967; Harborne and Mabry, 1982; Swain, 1965).

Flavonoids in Rosaceae are reviewed by Harborne (1967) and Hrazdina (1982). Among anthocyanins, cyanins are the most common to occur, followed by peonin and pelargonin. There is a complete absence of delphinin in the family. The glycosylation occurs at the 3 and/or 5 positions. Flavonols having 3', 4',and 5' trihydroxy substitution (trihydroxy flavonol; myricetin) are usually absent in this family. Kaempferol and quercetin are usually present in many species with various glycoside patterns.

The phenolic compounds in the leaves of genus _Potentilla_ were examined by Bate-Smith (1961). Forty-one species were examined chromatographically. Quercetin and kaempferol (flavonols) were present in almost all species. A glycoside kaempferol was present only in a few species. Cyanidin, probably a hydrolysis product of proanthocyanidin (Haslam, 1982) was found in most species. Unidentified dihydrochalcones were also found in some species. Phenolic compounds other than the flavonoids present were ellagic acid (an acid hydrolysis product of ellagitannin), p-coumaric, caffeic, ferulic and cinnapic acids (phenylcarboxylic acids with various substitution), with a decrease in frequency of presence in the order listed.

P. fruticosa was reported to contain quercetin, ellagic acid, cyanidin, caffeic, sinapic, and ferulic acids and kaempferol in decreasing amounts in the order listed (Bate-Smith, 1961). A more recent report by Fedoseeva (1980) supported the above finding. In the epigeal part of the plant, the author also found (-)-epicatechin gallate, (±)-catechin, (-)-epicatechin, (-)-epigallocatechin, and (-)-epigallocatechin gallate. The (±)-catechin and (-)-epicatechin are procyanidin biosynthesis precursors (Haslam *et al*, 1977) or acid degradation products of procyanidin (Haslam, 1977).

Cyanidin was not reported by Fedoseeva (1980), however (-)-epicatechin, (+)-catechin, and cyanidin are acid degradation products, differing in oxidation state (Haslam, 1977), thus they may have been the same compound *in vivo*. P-coumaric acid, not previously reported, was detected by Fedoseeva (1980).

The separation and identification of flavonoids (aglycone and glycone) became less difficult with the aid of paper chromatography (Harborne, 1958a, and 1959; Harborne and Sherrat, 1957), along with the spectral characters of the pigments (Harborne, 1958a; Jurd, 1962). Large scale separations are best carried out using column chromatography (Anderson and Sowers, 1968; Hostettman and Hostettman, 1982; Hrazdina, 1970; Mabry et al, 1970; Markham, 1982). The requirement for analyzing very small quantity samples and better resolution made thin layer chromatography (TLC) an alternative to paper chromatography (Asen, 1965; Jay et al, 1975; Markham, 1982; Nybom, 1964; Stahl, 1969). High-pressure liquid chromatography offers much better separation than TLC in qualitative and quantitative assays (Asen, 1979; Brenneisen and Steinegger, 1981; Hostettman and Hostettman, 1982; Markham, 1982). Other techniques include paper electrophoresis, high-pressure TLC, centrifuge TLC, gas-liquid chromatography, and droplet counter-current chromatography (Hostettman and Hostettman, 1982; Markham, 1982).

2.1.2 Carotenoids

Carotenoids are fat soluble pigments, the majority of which are tetraterpenes with a 40 carbon-skeleton (Weedon, 1971). There are two types of carotenoids; carotenes which are hydrocarbons and oxygenated carotenoids which are xanthophylls. The number of carotenes is relatively small and most carotenoids, about 300 now known, are xanthophylls. Most carotenoids are brightly coloured in yellow to red.

Carotenoids are synthesized through the isoprenoid pathway; a general pathway for terpenes (Goodwin, 1971)

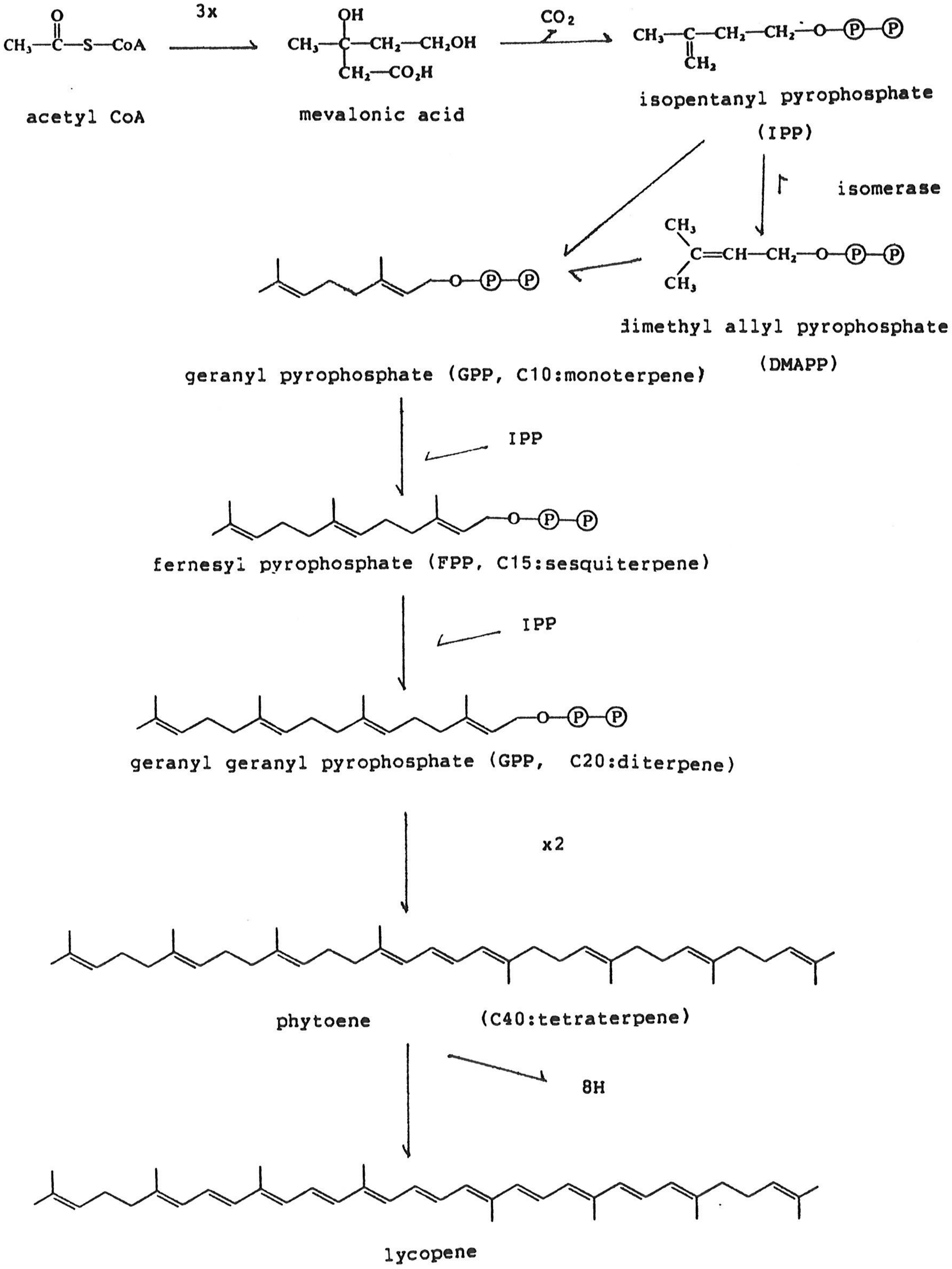

Figure 2: Biosynthesis of Carotenoids

(Figure 2). Three units of acetyl CoA are joined to produce mevalonic acid. Upon the loss of one carbon unit, isopentanyl pyrophosphate is formed. This is the fundamental five carbon-terpenoid precursor. The 10 carbon molecule, geranyl pyrophosphate, is formed by a transfer of one isopentanyl pyrophosphate to dimethyl allyl pyrophosphate, and subsequent transfers of two more isopentanyl pyrophosphate units will yield geranyl geranyl pyrophosphate, 20 carbons. The first 40 carbon compound is phytoene, formed by head to head condensation of two geranyl geranyl pyrophosphate units. A step-wise dehydrogenation of phytoene yields lycopene, which is a cyclization precursor in most carotenoids. Other carotenoids can be derived from lycopene by hydrogenation, dehydrogenation, cyclization, oxidation, or any combination of these processes.

Carotenoids are found throughout the plant kingdom (Weedon, 1971). Within the higher plants, they are present in leaves, although the colour is often masked by chlorophylls; flowers, fruits, and other parts of plants.

The flowers containing carotenoids include various roses (Valadon and Mummery, 1968a and 1969), various compositae species (Valadon and Mummery, 1967), Geum species (Valadon and Mummery, 1968a), narcissus, daffodil, tulip (Valadon and Mummery, 1968b), chrysanthemum (Stickland, 1972), Strelitzia reginae (Simpson et al, 1975), and saffron (Pfander and Rychener, 1982). Tomatoes (Umeil and Gabelman, 1971), apples (Valadon and Mummery, 1967), various berries (Valadon _et al_, 1975), and ornamental gourds (Osman _et al_, 1984) are some of the fruits containing carotenoids. They are also found in carrot roots (Mok _et al_, 1976; Umeil and Gableman, 1971). There usually are 10 to 20 types of carotenoids present in each specimen.

Schmid and Polaczek-Wittek (1939) reported that _Potentilla erecta_ contained ß-carotene, lutein and two additional compounds, perhaps zeaxanthin and flavoxanthin. Two species of _Potentilla_; _P_. _reptans_ and _P_. cv Woodbridge Gold; investigated by Valadon and Mummery (1968a) contained in common; ß-carotene, 5,6-monoepoxy-α-carotene, 5,6:5',6'-diepoxy-α-carotene, 5,6:5',6'-diepoxy-ß-carotene, mutachrome, trans-luteinepoxide, lutein, and flavoxanthin. They also contained nine other carotenoids at smaller quantities. The two species contained over 30 percent in 5,6:5',6'-diepoxy-α-carotene and 20 to 30 percent in mutachrome. A relatively high amount of α-carotene epoxy derivatives may be a characteristic of this genus. Total carotenoids were 3.70mg/g and 2.19mg/g dry weight, respectively. There have been no reports on _P_._fruticosa_.

Isolation and identification methods are outlined by Liaaen-Jensen (1971), Davies (1965) and Goodwin (1955). Chromatographic identification methods include column, paper, thin-layer, and gas chromatography (Bolliger and Konig, 1969; Liaaen-Jensen,1971). More recent high-performance liquid chromatography procedures are described by Eskins et al (1977), Pfander and Rychener (1982), and Stancher and Zonta (1982). Purified materials are also analyzed by absorption spectroscopy by ultraviolet and visible light, infrared spectroscopy, nuclear magnetic resonance and mass spectroscopy (Vetter et al, 1971).

2.2 ENVIRONMENTAL EFFECTS ON FLOWER COLOUR

2.2.1 Anthocyanins

Flower colour variation within a species arises through the effect of genetic and/or environmental factors. Although genetic control over the types of pigments present determines the colour expression of flowers in many cases (De Vries et al, 1974 Grant, 1975; Harrison and Stickland, 1974 and 1978; Marshall, 1975; Stickland and Harrison, 1974 and 1977), the wide variation of flower colours derived from six commonly occurring anthocyanins cannot be accounted for by the presence of these pigments *per se* (Asen, 1976). The six types of pigments, in purified forms, usually represent the colours of scarlet (pelargonin), magenta (peonin and cyanin), and purple (delphinin, petunin, and malvin) (Harborne, 1958a).

Other factors such as the concentration and molar ratio of the pigments (Asen et al, 1971; Biran et al, 1974a and 1974b; Chriki and Harborne 1983; Harvey, 1971), metal chelate complexes (Asen et al, 1969; Yasuda, 1970), pH (Asen et al, 1971 and 1975; Biran et al, 1974a; Stewart et al, 1975), and co-pigmentation (Asen, 1976; Asen et al, 1972 and 1975; Chen and Hrazdina, 1981; Yazaki, 1976) play an important role in determining flower colour.

Environmental factors that affect flower colour may include nutrient status, plant water status, light and temperature. In many cases, light and temperature are the most important factors (Harborne, 1965).

The effect of light on anthocyanin synthesis is reported by many authors (Drum and Mohr, 1974; Drum et al, 1975; Duke et al, 1976; Grill and Vince,1964; Hrazdina and Parsons, 1982; Knoblock et al, 1982; Ku and Mancinelli, 1972; Lange et al, 1971; Mancinelli and Rabino, 1975; Mancinelli et al, 1974, 1975, and 1976; Pecket and Bassim, 1974, Smith, 1975).

Anthocyanin production in marigold leaves increased with higher quantum flux density (Armitage and Carlson, 1981). Lack of light on the leaves resulted in an inhibition of the pigmentation of Baccara rose flowers (Biran and Halevy, 1974a), and shading the whole plants decreased the level of pigments (Biran and Halevy , 1974b).

The role of ultraviolet light in anthocyanin synthesis is reported by Chalmers and Faragher (1977), in which the exposure of apple skin to ultraviolet light promoted anthocyanin accumulation. *Primula malacoides* flowers required ultraviolet light between 300 to 360nm for anthocyanin synthesis (Kashiwagi et al, 1977).

An inverse relationship between average temperature and anthocyanin formation in McIntosh apples was reported by Creasy (1968). The author also reported that a low temperature treatment was inductive in anthocyanin formation in Euonymus leaves (1974), an example of autumn colouration triggered by temperature. Synthesis of anthocyanin in the foliage of Tagetes patula cv. Petite Yellow was greatest at a low day temperature of 10°C (Armitage and Carlson, 1981). Rutland (1968) reported that higher temperature decreased anthocyanin in Chrysanthemum morifolium cv. Orchid Queen flowers with the greater effect due to night temperature treatments. Anthocyanin production in cultured C. morifolium cv. Fandango was highest at a low temperature of 15°C (Stickland, 1974a).

Temperature treatment on various parts of Baccara rose yielded a result in which cooling the buds caused the most enhancement of pigmentation (Biran and Halevy, 1974a). Heat stress applied to whole plants or to flower buds only resulted in a decreased level of pigments (Biran and Halevy, 1974b).

Reduction of boron, molybdenum, or zinc in a growth medium resulted in an increase in anthocyanins in Spirodela oligorrhiza, whereas the effect of copper was less apparent

(Thiman and Edmondson, 1949). Soil nutrient deficiencies of nitrogen, phosphorous, or sulphur resulted in anthocyanin formation in many plants (Salisbury and Ross, 1978). Low CO_2 concentration decreased anthocyanin content in Baccara rose flowers (Biran _et al_, 1973). Sugar added to the _Spirodela oligorrhiza_ culture medium had a marked promotion on anthocyanin formation, although various sugars and sugar alcohols resulted in different effectiveness (Thiman and Edmondson, 1949; Thiman _et al_, 1951). A study on _Chrysanthemum morifolium_ cv. Fandango florets supported the above findings with the highest anthocyanin content at four percent sucrose in the culture medium (Stickland, 1974a). Sugar concentration in _C. morifolium_ cv. Orchid Queen petals, however, was negatively correlated with anthocyanin concentration (Rutland and Seawright, 1973).

Anthocyanins are reported to have increased in detached leaves of _Quercus coccifera_, _Q. ilex_ and _Q. rubur_ with decreasing relative water content (Spyropoulos and Mavrommatic, 1978), although the response varied by an interaction with temperature (Spyropoulos and Lambiris, 1978).

2.2.2 Carotenoids

Relatively little is known about flower colour change due to carotenoids.

Carotenoids occur in the chromoplasts, which arise from chloroplasts in fruits (Goodwin, 1966). Disappearance of chlorophylls was followed by rapid carotenoid synthesis in the plastids. A similar report was made by Simpson *et al* (1975) for the sepals of *Strelitzia reginae*.

Carotenoid synthesis is known to require light. A fungus, *Verticillium agaricuinum*, grown in light, produced carotenoids eight times as great as in the dark control (Valadon *et al*, 1982). Dark grown mung bean seedlings contained a small amount of carotenoids and the total carotenoids greatly increased under illumination, with a larger increase in xanthophylls than carotenes (Valadon and Mummery, 1982). The effect of light on wheat seedlings was less stimulatory (Wolf, 1963). The light grown seedlings contained only twice the amount of carotenoids as the dark grown. However the effect varied on each carotenoid, with the larger increase of carotenes.

Wheat leaf sections accumulated less plastid carotenoids when exposed to polyethylene glycol induced water stress at -9 to -14 bars (Dysen and Freeman,1974). This effect was partially overcome by hormone applications of benzyladenine and gibberellic acid (Duysen and Freeman, 1976).

Carotenoid synthesis in tomato fruit was affected by temperature (Goodwin, 1966). There was much higher total carotenoids at lower (23.5°C) temperature than the higher (32°C) temperature. However the effect varied for each type of carotenoid depending upon genotype. An opposite effect of temperature was observed in Chrysanthemum morifolium cv. Fandango (Stickland, 1974a). The cultured florets formed the highest amount of carotenoids at 30°C, seven times more than florets cultured at 6°C.

2.3 PETAL NUMBER

The physiology of flowering is mainly focused on initiation and development of flowers (Bernier et al:1981). Petal number is a secondary aspect of flowering and is generally determined by genetic control.

Petal number in Baccara rose was increased by low temperature treatments (12°C), resulting in "bullheads" (Moe, 1971). The treatment was most effective when applied during the early stages of development, during floral part differentiation. Similar temperature (12 to 15°C) applied before the differentiation of floral parts often resulted in blind shoots, an abortion of flower buds.

In carnation, low temperature treatment (5°C) resulted in an increase in petal number through production of secondary growing centers (Garrod and Harris, 1974). A similar result was obtained through applications of gibberellin or indoleacetic acid. Localized high temperature treatment also produced increased petal numbers in these plants. However these extra-petals were produced directly on the receptacle and the hormone inductive to this condition was kinetin. The increase of petal number in the former was greater than the latter treatment.

Genetically double flowering plants became phenotypically single flowering by high temperature treatment in freesia (Baer and Kho, 1971). A similar reversion to single flowers from double flowers by warm greenhouse conditioning was cited for *Kerria japonica* (Reynolds and Tampion, 1983).

Chapter III

MATERIALS AND METHODS

3.1 FLOWER COLOUR

3.1.1 Pigment Analysis

Preliminary pigment analysis was carried out to identify the types of pigments involved in the expression of flower colour.

3.1.1.1 Anthocyanins

The methods used by Harborne (1958a) and Nybom (1964) were adapted for this experiment. Fresh petal samples were extracted with a small volume of solvent consisting of one percent (v/v) concentrated HCl dissolved in methanol. The extract was centrifuged at 12,000 rpm for 15 minutes to remove any plant material. The supernatant was pipetted out, then without further concentration, the crude extract was directly applied several times to 3MM Whatman chromatographic paper (18 cm x 23 cm) in a band 2 cm from the lower edge. The paper was developed ascendingly in chromatographic tanks containing n-butanol: acetic acid: water (4:1:5, v/v, top layer). The red coloured bands, after air drying, were cut out and the pigments were re-extracted in the same solvent overnight. The extract was microfuged for five minutes to remove cellulose fiber. The extract was spot applied many times on cellulose thin layer plates (Eastman Chromatogram sheet, 20 cm x 20 cm). The plates were developed in one direction in the tanks containing water: acetic acid: 12N HCl (82: 15: 3, v/v), n-butanol: 2N HCl (1:1, v/v, top layer), or 1 percent HCl aq.

The pigment extracts from the paper chromatograms were hydrolyzed to remove sugar groups to obtain aglycones, (anthocyanidins). Five mls 1N HCl was added to approximately the same volume extract and the acid hydrolysis was carried out in a boiling water bath for one hour with N_2 stream. N_2 stream was used to minimize enzymatic or nonenzymatic polymerization of phenolics. The cooled solution was partitioned twice with one ml n-amyl alcohol. The combined amyl alcohol extract was washed three times with five mls water to remove acid and sugars. The extract was spotted on cellulose thin layer plates and developed in Forestral solvent (acetic acid: concentrated HCl: water, 30:3:10, v/v) or in two directional chromatography using n-amyl alcohol: concentrated acetic acid: water (2:1:1, v/v) and concentrated formic acid: concentrated HCl: water (10:1:3, v/v).

The acid aquatic layer containing sugars was concentrated in a heated water bath with an air stream. The concentrate was spotted on 3MM Whatman chromatographic paper with known sugars. The chromatogram was developed in ethyl acetate: pyridine: water (10:4:3, v/v) descendingly. The chromatogram was stained with silver nitrate in acetone, NaOH in ethanol, and then aqueous sodium thiosulfate to visualize the spots.

3.1.1.2 Carotenoids

The method used by Goodwin (1955) was adapted for the extraction of carotenoids in the petals. The pigments were extracted from fresh petals with methanol several times. Then with methanol: diethyl ether (1:1, v/v) until there was no trace of colour. The combined extracts were concentrated in vacuo, and diluted with an equal volume of diethyl ether. Partition was carried out upon the addition of water. The upper layer containing carotenoids was washed with water to remove any trace of methanol. The lower layer was reextacted with diethyl ether. The combined, washed extracts were concentrated and dried.

The dried pigments were redissolved in ethanol. Sixty percent KOH aq. was added at the ratio of one ml KOH aq. to 10 ml ethanol. The mixture was stored overnight (12 to 16 hours), in N_2, in the dark, at room temperature for saponification of natural fats. The saponified solution was diluted with three to four times the volume of water. The pigments were reextracted with diethyl ether several times until no colour was detected. The combined extracts were washed with one half volume of tepid water several times to remove the base. This was checked with litmus paper.

3.1.2 Environmental Effects

Based on the field observation that flower colour was more intense during spring and fall, temperature and moisture factors were selected as the main environmental factors. A field experiment was conducted during the summer of 1983 using existing taxa at the university to determine the main environmental cause of flower colour change. Taxon (pl. taxa) is used to include categories such as species and varieties (Benson, 1959) as well as native populations, cultivars and selection lines in this paper. The plants were placed in rows 1.5 m apart and spaced at 60 cm intervals within the rows. There are two plants for each taxon. During the summer of 1983, no fertilizer was applied and irrigation was applied once during a very dry period.

There were two types of observations made for each taxon: one a general flower colour classification and two petal pigment concentrations at anthesis.

3.1.2.1 General Evaluation

One hundred sixty-two taxa were assessed once a week for flower colour, and flowering period during the growing season.

Each flower was classified according to the following guideline.

Classification of flower colours (Figure 3)

acyanic colours

1	white
2	creamy white
3	cream
4	lemon cream
5	lemon
6	light yellow
7	yellow
8	dark yellow

cyanic colours

orange	O-1	O-2	O-3	O-4	O-5
pink	P-1	P-2	P-3	P-4	P-5
salmon	S-1	S-2	S-3	S-4	S-5

The flowering period was also recorded for the taxa.

3.1.2.2 Environmental Factor Measurement

Temperature was recorded at the weather station at the University of Manitoba experimental plot (Minneapolis-Honeywell, Brown Instruments Division Regulator Co. Ltd.). Daily maximum and minimum temperatures were obtained from the recorded chart. Weekly precipitation was obtained using a rain gauge. Plant water potential was measured using a pressure bomb (PMS instrument Co.) (Scholander et al, 1965).

Figure 3: Classification of flower colours

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3.1.2.3 Pigment Concentrations

Ten cultivars and selection lines were chosen to examine the effect of environmental factors on anthocyanin and carotenoid concentrations in the flower petals. The taxa used were;

Daydawn	salmon
Friedrichseni	lemon
Glabra	light yellow
Orangeman	orange
Sunset	orange
Tangerine	orange
UM7513	salmon
UM7904	orange
UM7911	pink
UM8105	orange-red

The above cultivars were used in the breeding program at the University and the selection lines are some of the advanced breeding lines.

The pigment concentrations were measured spectrophotometrically. The method used by Stickland (1972) was adapted for the experiment, as detailed in the subsequent sections.

3.1.2.4 Anthocyanins

Five flowers from each taxon were collected at random once a week. The physiological age of the petal tissue used for the pigment extraction was standardized using flowers at about anthesis. Petals were removed from each flower and anthocyanins were extracted in two mls one percent (v/v) concentrated HCl dissolved in methanol in the dark at two to 4°C for two days. The absorbance was measured at 525 nm on a spectrophotometer (Hitachi 100-40). Peak cyanin absorbance of the crude extract of these taxa occurred at this wavelength and the standard deviation was relatively small compared to other methods.

The pigment concentration was calculated using the molar extinction coefficient ($\epsilon = 2.95 \times 10^4$, Stickland, 1972) and expressed in mg pigment/g fresh weight.

Anthocyanin concentration was corrected for carotenoids since degradated carotenoids in acid solvent were found to have some absorbance at 525 nm (A_{525}). A regression equation was obtained between independent (A_{470}) and dependent (A_{525}) of carotenoids (Figure 4). This regression equation of absorbances, $A_{525} = 0.1600 \times A_{470}$, was modified for the pigment concentrations using $\epsilon = 2.95 \times 10^4$ for anthocyanins and $\epsilon = 1.20 \times 10^5$ for carotenoids (Stickland, 1972).

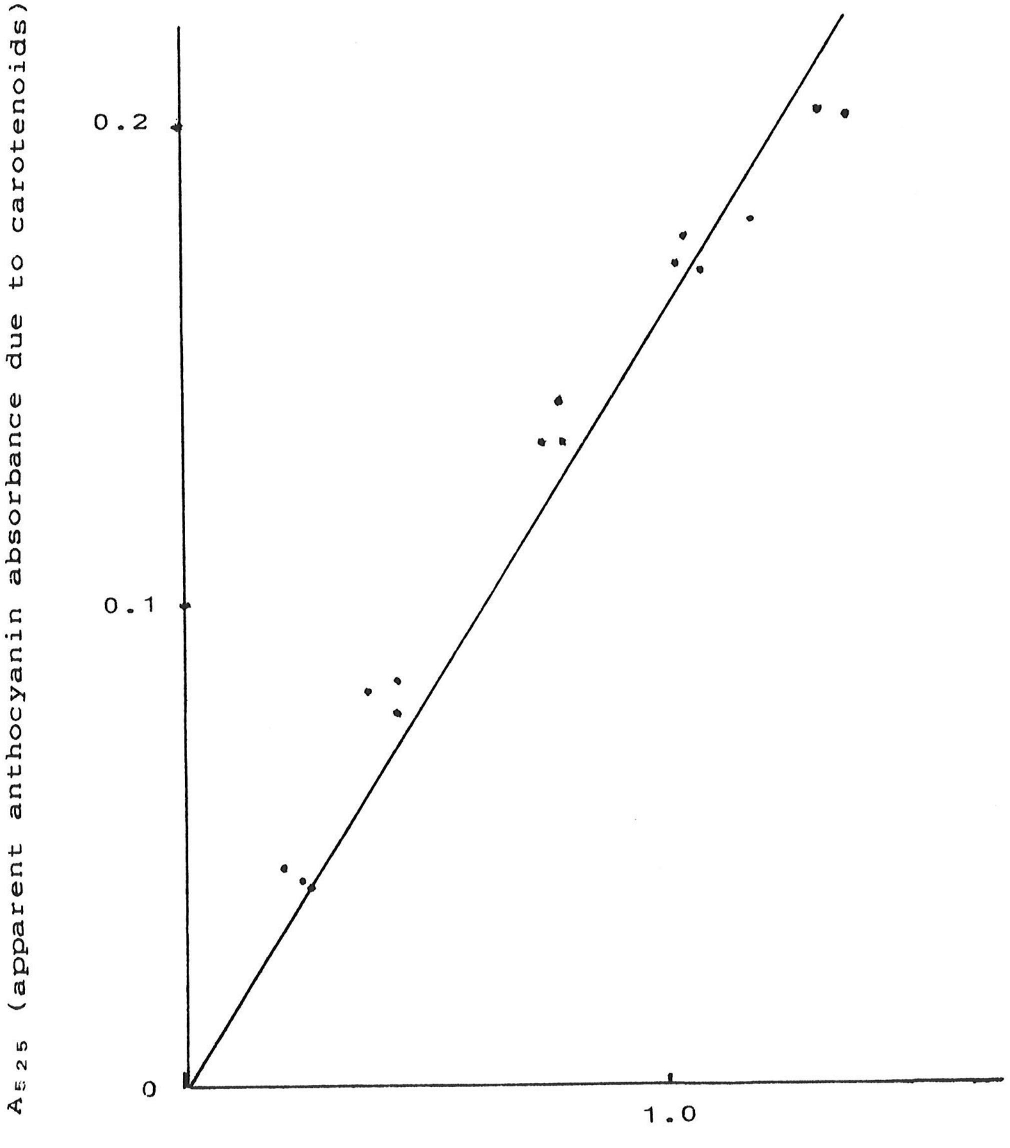


Figure 4: The relationship between apparent anthocyanin absorbance due to carotenoids and carotenoid absorbance

Apparent anthocyanins due to carotenoids (mg/g fwt)

$$= \frac{\dfrac{500}{2.95 \times 10^4} \times 1000}{\dfrac{536}{1.20 \times 10^5} \times 1000} \times 0.1600 \times \text{carotenoids (mg/g fwt)}$$

$= 0.60 \times$ carotenoids (mg/g fwt)

Actual anthocyanin (mg/g fwt)

= apparent anthocyanins (mg/g fwt)
 - apparent anthocyanins due to carotenoids (mg/g fwt)

= apparent anthocyanins (mg/g fwt)
 - $0.60 \times$ carotenoids (mg/g fwt)

3.1.2.5 Carotenoids

Samples for carotenoids were collected the same as for anthocyanins. The pigments were extracted from the petals using two mls petroleum ether in methanol (20:80, v/v), in the dark at two to 4°C for two days. The absorbance was measured at 470 nm. Pigment concentration was obtained using $\epsilon = 1.2 \times 10^5$.

3.1.3 Temperature Effects

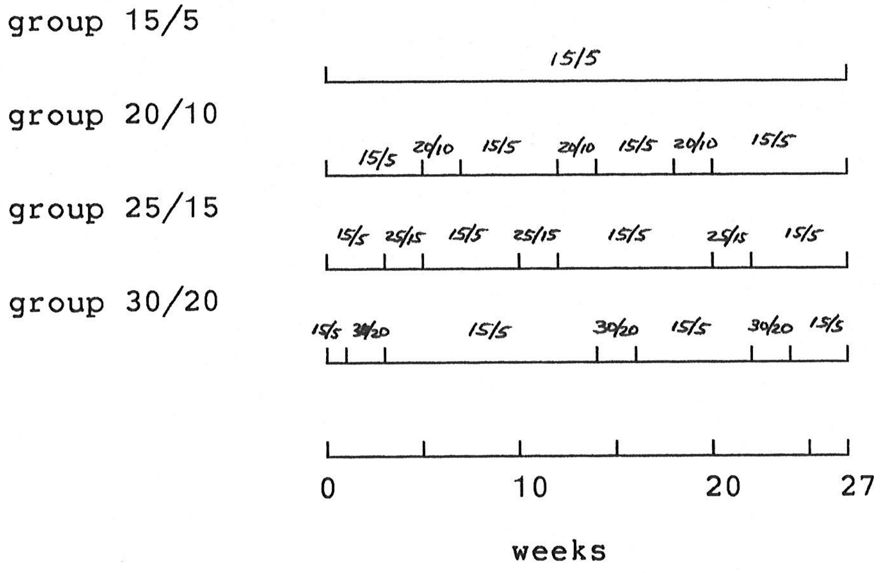
The effect of temperature on flower colour was further investigated in a controlled environment.

Cuttings were made from the field plants during the fall of 1982, treated with a rooting hormone (Stim Root No.2, Indole-3-butyric acid 0.4 percent), and rooted in 5 cm peat pots in a medium of peat moss and vermiculite (1:1, v/v). The pots were placed in a mist chamber until the cuttings were rooted in about a month. Rooted plants were transferred into 10 cm plastic pots with a soil mixture of loam: sand: peat (1:1:1, v/v). The plants were grown into larger sized plants in 25 cm clay pots in greenhouses and growth cabinets for about a year in order to ensure a continuous flower supply. The plants were watered as required and fertilized with 10-52-10 or 20-20-20 once a week.

Experiments were conducted in three growth cabinets having 16 hours daylength with light from fluorescent light tubes (Sylvania, FR96T12/GRO/WS/VHO/235 and F48T12/GRO/WS/VHO). Two larger sized cabinets (Econaire Systems Ltd., GRW-36) were used for the control temperature. A smaller cabinet (Econaire Systems Ltd., E-15) was used for the treatment temperatures. The three cabinets had similar environmental conditions other than temperature. The relative humidity was 60 to 70 percent (Sling Psychrometer, Bacharac Instrument). The light intensity at the plant level was at 500 to 600 $\mu E/m^2/sec$ (quantum photometer, LI-COR Inc. model LI185B).

Two selection lines were used for the experiment. There were four temperature treatment groups including the control, with four plants in each group for each selection line (32 plants). The control day and night temperatures were 15^0C and 5^0C, respectively, with 2^0C per hour temperature succession. The three treatments were day/night temperatures of 20/10°C, 25/15°C, or 30/20°C with 2°C per hour temperature succession. The temperatures were set at the top of plant canopy using a thermistor (Atkins Technical Inc.). Maximum temperature deviation was ± 0.5°C.

Plant arrangement was randomized within the cabinets. The treatments were applied by moving the plants from the control cabinets into the treatment cabinet for two weeks, then moving back to the control cabinets. Each treatment group received temperature treatments during the 27 week treatment period in the following order;



3.1.3.1 Pigment Concentrations

The two selection lines used were UM7904 and UM7911. Four flowers were collected from each plant on Mondays, Wednesdays, and Fridays and the extra flowers were picked off to ensure that the sample flowers were at the same flowering stage of anthesis.

Two flowers from each plant were used for anthocyanin concentration determinations and the others to determine the carotenoids. There were eight flowers per temperature treatment per selection line per day. The flowers were kept separated for each plant. The pigment concentrations were determined spectrophotometrically as discussed in 3.1.2.4 and 3.1.2.5.

3.2 FLOWER BUD DEVELOPMENT AND PIGMENT SYNTHESIS

Flower bud development and pigmentation of petals were recorded for UM7904 in a growth cabinet (Econaire Systems Ltd., GRW-36). The experiment was conducted on plants at the end of two week temperature treatments at 15/5°C and 30/20°C day/night temperatures. The buds were divided into eight developmental stages.

stage	description	
1	bud diameter <2.5 mm	↑ bracts covering the petals
2	bud diameter 2.5 to 4.0 mm	
3	bud diameter 4 to 6 mm	bracts open
4	bud diameter > 6 mm (before opening)	↓
5	opening	
6	anthesis	
7	senescence 1 (anthers start to brown)	
8	senescence 2 (anthers off)	

Flower buds were sampled at random within the population and bud diameter and petal weight were obtained. These samples were used for anthocyanin and carotenoid concentration determinations. The procedures were the same as before and the concentrations (mg pigment/g fresh weight) and total pigment (mg pigment/ bud) were calculated.

3.3 PETAL NUMBER

3.3.1 Environmental Effects

A field experiment was conducted examining the change of petal numbers of the existing taxa. A general flower characteristic observation on all taxa and more detailed examination of petal number on four cultivars and selection lines were carried out.

3.3.1.1 General Evaluation

The cultivars and selection lines were examined in the same manner as for flower colour. The petal number was recorded as either single (5) or having more than five petals (EXP).

3.3.1.2 Petal Number Change

Four cultivars and selection lines were selected for examining petal number change. These were;

Hersi	double white
Sundance	double cream
UM7901	double yellow
UM8102	double white

Samples of 20 flowers obtained at random from each cultivar and selection line once a week were recorded for petal number per flower.

3.3.2 Temperature Effects

The effect of temperature on petal number was further investigated in growth cabinets in the same manner as for flower colour. Two selection lines, UM7901 and UM8102, were used. Five flowers were collected from each plant on Mondays, Wednesdays, and Fridays and the petal number per flower was recorded. The extra flowers were picked off to ensure the same physiological age of flowers. There were 20 flowers per treatment per selection line per day. In this experiment, the total number of open flowers (anthesis to senescence) per plant was recorded on each sampling day as well.

Chapter IV

RESULTS AND DISCUSSION

4.1 FLOWER COLOUR

4.1.1 Pigment Analysis

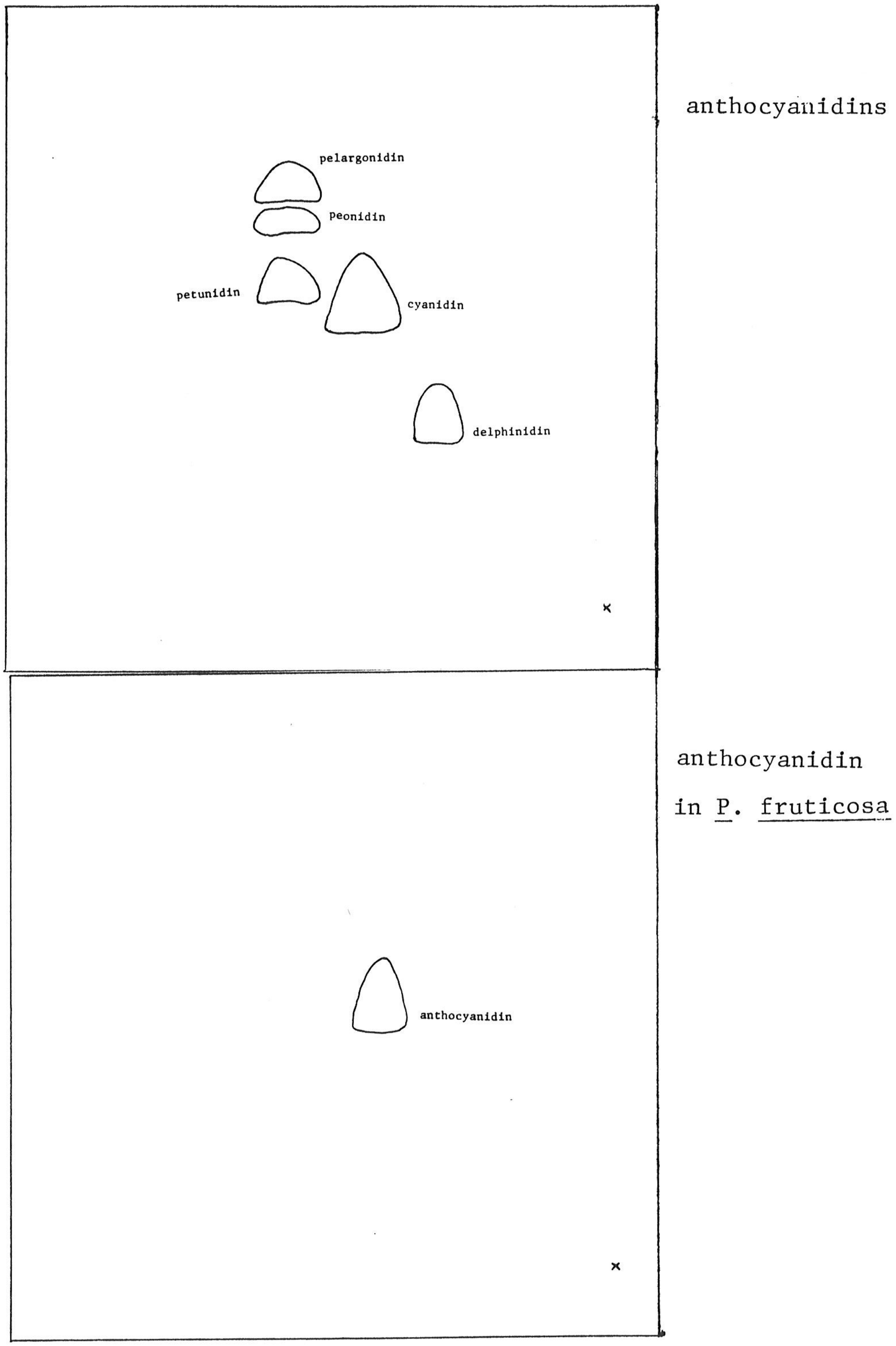
4.1.1.1 Anthocyanins

Anthocyanins were present in orange, pink and salmon coloured flowers respectively in decreasing amount. Acyanic flowers did not contain detectable anthocyanins. Further analytical work was conducted on UM7904 and UM7911.

Preliminary chromatographic anthocyanidin identification strongly indicated that the pigment in UM7904 and UM7911 was cyanidin (Figure 5).

There appears to be two cyanins in UM7904 and one in UM7911 (Figure 6). The Rf values indicate that UM7904 cyanin-1 is probably 3-diglucoside and cyanin-2, the larger quantity of the two, is 3-monoglucoside or 3-monogalactoside. The cyanin in UM7911 is probably the same as the cyanin-2 of UM7904.

Paper chromatography for the sugars indicated that cyanin-1 contained glucose and cyanin-2 contained glucose,


anthocyanidins

anthocyanidin in *P. fruticosa*

Figure 5: Thin layer chromatograms of anthocyanidins in authentic samples and in *P. fruticosa*

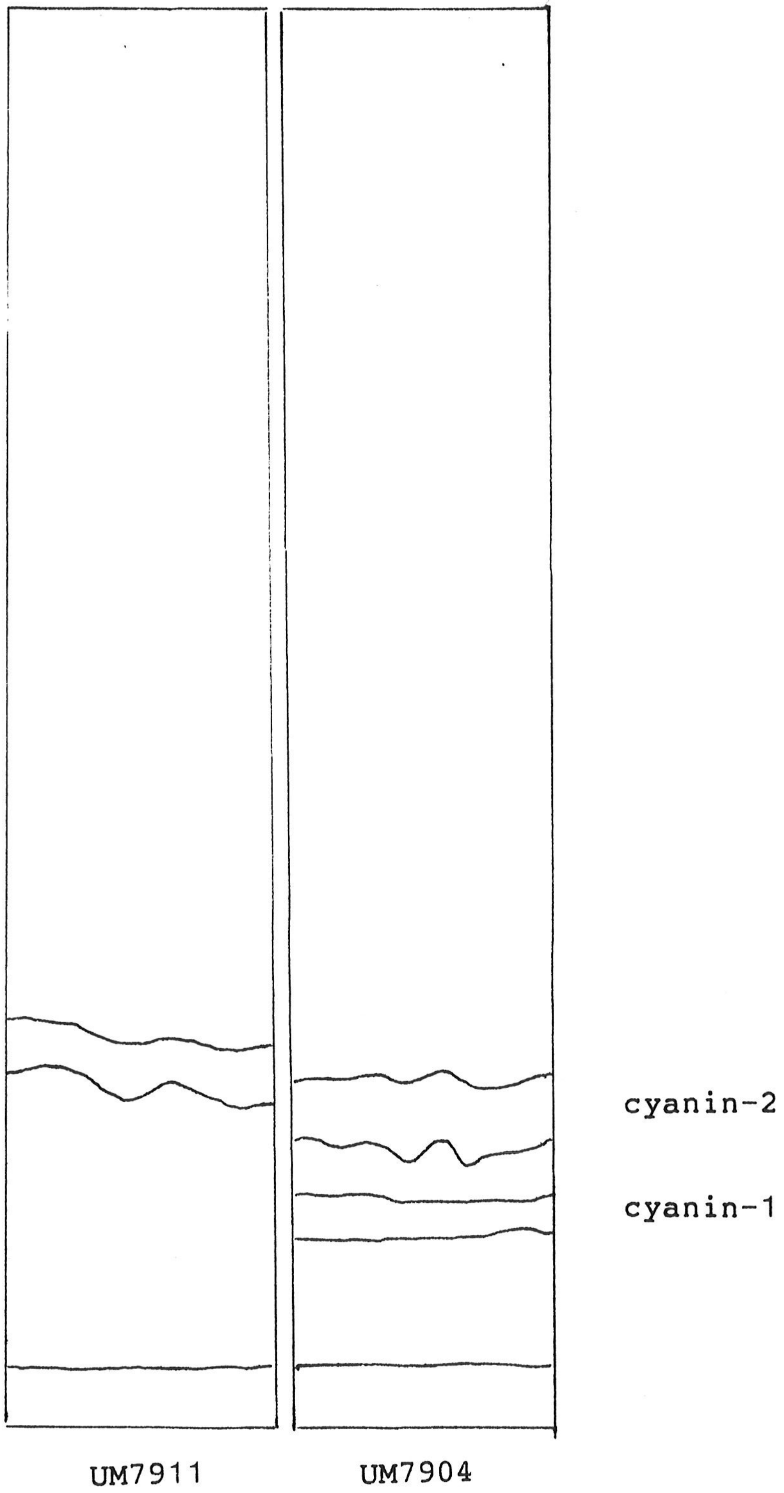


Figure 6: Paper chromatograms of anthocyanins in *P. fruticosa* selection lines

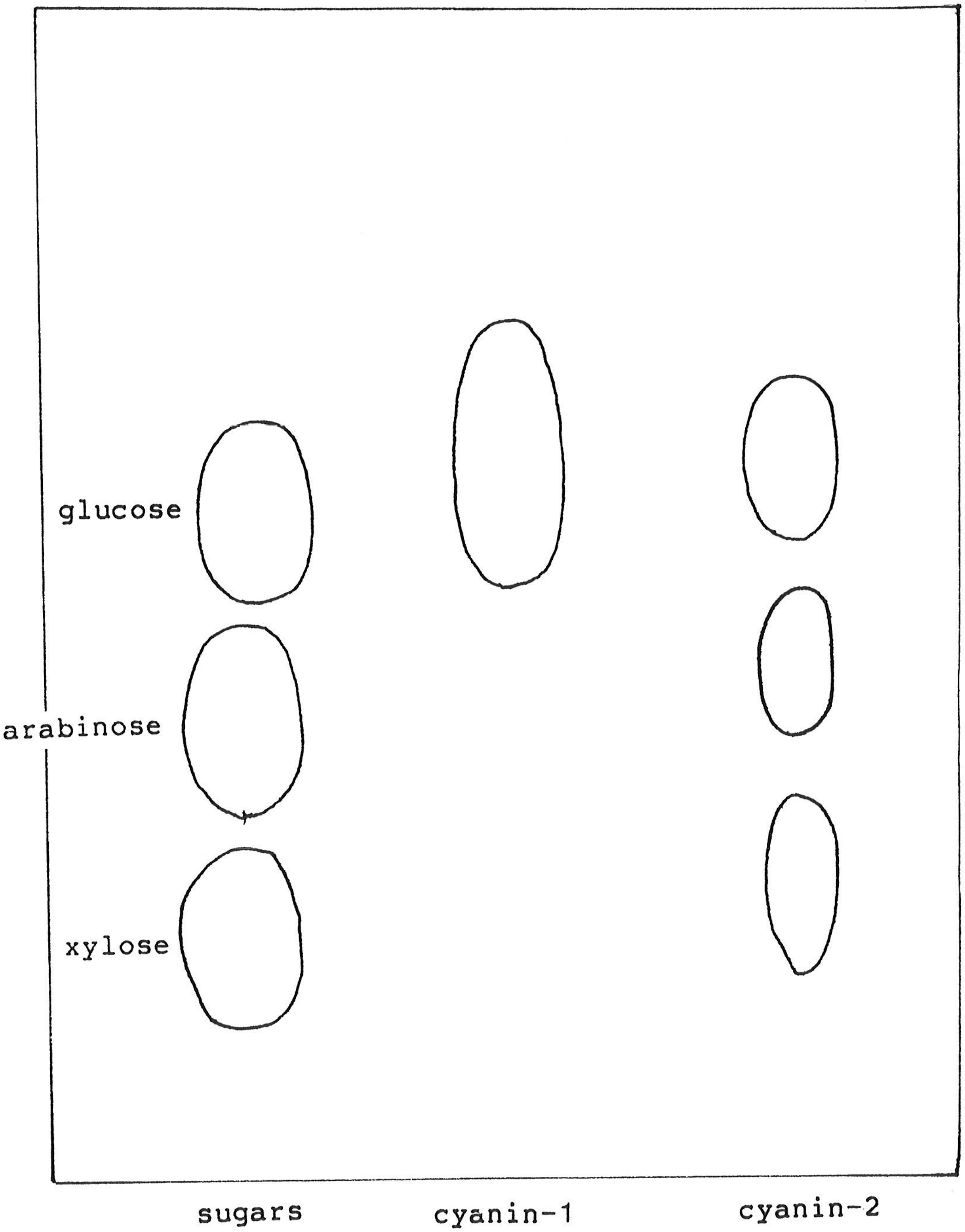


Figure 7: Paper chromatogram of authentic sugars and sugars in anthocyanins of *P. fruticosa*

arabinose and xylose (Figure 7). Since arabinose is probably an artifact (Harborne and Sherratt, 1957), cyanin-2 is probably 3-xyloglucoside. Co-chromatography with authentic samples and spectral characterization will be required to confirm these results.

The usual procedures for anthocyanin analysis are relatively simple (Harborne, 1958a; Nybom, 1964), however the crude extracts of *P. fruticosa* readily formed brown tannin-like substances upon concentration *in vacuo* unlike many other plant species. Although the tannin-like substance was not confirmed, precipitation occurred upon the addition of serum albumin which is characteristic of a tannin (Haslam, 1982). The plant is known for very few insect problems and this further suggests a high tannin content. The presence of proanthocyanins, a group of tannins, has been reported in the vegetative part of the plant (Bate-Smith, 1961; Fedoseeva, 1980).

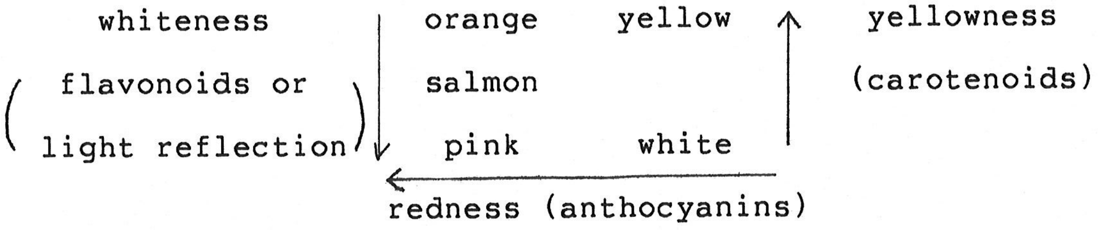
The presence of two cyanins in the two selection lines examined indicates that *P. fruticosa* flowers synthesize a few types of cyanins. Although there are over 200 known anthocyanins, most plant species contain only a few (Hrazdina, 1982).

There was a wide pale yellow band beyond the cyanins on the paper chromatograms. These are probably yellow flavonoids (Harborne, 1959).

4.1.1.2 Carotenoids

Carotenoids were present in yellow, orange, and salmon coloured flowers respectively in decreasing amount, and absent or not readily detectable in white or pink flowers. The intensity of yellow colour of the extract visually corresponded to the yellow colour in the flowers, thus the yellow flower colour is mostly due to carotenoids and not due to yellow flavonoids.

The colour variation within the species is mainly due to the concentration differences of the two types of pigments; anthocyanins and carotenoids. The yellow flowers contained carotenoids and decreasing the amounts of carotenoids produced softer shades of yellows until they were absent in the white. The orange flowers contained anthocyanins and carotenoids. The pink flowers contained little or no carotenoids, but appeared pink instead of red or magenta (colour of cyanin) due to the white colour as a result of the presence of white flavonoids or by reflection of light (Stickland, 1974b). The salmon flowers contained all three types of colour elements, red, yellow, and white. The above variation of flower colour may be presented diagramatically as follows.



4.1.2 Environmental Effects

4.1.2.1 General Evaluation

There were 162 taxa evaluated according to the classification of flower colours described. These were divided into; 22 orange, 5 pink, 8 salmon, 16 white, 7 creamy white, 7 cream, 11 lemon cream, 22 lemon, 10 light yellow, 32 yellow, 21 dark yellow and one unknown due to no flowers (Appendix A).

The number of flowering days ranged from 50 days to 127 days. Flowering started June 7th and the last flowering date recorded was October 11th, the last sampling date before the killing frost. The overall mean was 91.4 days. The mean for the named cultivars and native plants was 94.3 days whereas the mean for the selection lines was 88.6 days. The mean number of flowering days varied among the colour groups, however only lemon cream (102.2 days) and salmon (79.1 days) differed significantly at Duncan's multiple range test (0.05) (table 1). There was a tendency toward a lower number of flowering days among the cyanic taxa.

Cyanic taxa were in general finer in foliage texture than acyanic taxa. It was reported that anthocyanin

TABLE 1

The mean flower period by flower colour groups

FLOWER COLOUR	FLOWER PERIOD (DAYS)*	
lemon cream	102.17	a
white	97.10	a b
yellow	97.10	a b
dark yellow	95.76	a b
pink	89.40	a b
lemon	89.32	a b
light yellow	89.00	a b
cream	86.86	a b
creamy white	86.00	a b
orange	82.18	a b
salmon	79.13	b

*Flower period values with the same letter did not differ significantly as determined by the Duncan's test (0.05) production of P. grandulosa had a highly significant correlation with leaf length (Grant, 1975). Plant growth appeared more vigorous among acyanic taxa.

4.1.2.2 Environmental Factors

When the disappearance of "redness" during the growing season was first observed, four environmental factors were considered for possible causation; light, nutrients, temperature, and moisture.

The decision not to study light effects was based on field observations. The most intense, cyanic flower colour occured at the beginning (long photoperiods and high solar irradiance) and end (short photoperiods and low solar irradience) of the floweriong period. There has been no report on the effect of the photoperiod on flower colour.

The nutrient factor was also eliminated due to a lack of deficiency symptoms. Most plants grew throughout the season with a continuous production of flowers.

Temperature and moisture factors were thus selected as the possible causes affecting flower colour.

Although there was a relatively cool spring in 1983, the temperature remained above normal from June through October (Annual Meteorological Summary, 1983), the month of the first killing frost (Appendix B). The month of June was slightly above normal, however in August, the average monthly maximum temperature was more than 6°C higher than normal, at 31.1°C. Overall, there were 43 days of daily maximum temperature over 30°C.

Precipitation was much below average (Appendix C). July to September total precipitation was 64 percent of a normal year (Annual Meteorological Summary, 1983). With the combination of high temperature and lack of moisture, many plants appeared to have suffered drought stress towards the end of the season.

4.1.2.3 Anthocyanins

Anthocyanin concentrations ranged from zero mg to 1.38 mg/g fresh weight among the taxa, and among dates (Figure 8). The pigment concentrations were higher at the beginning and at the end of the flowering season than July to August. The overall percent difference between maximum and minimum concentrations ranged from 84.6 percent to 100 percent and the concentration difference ranged from 1.296 mg /g fresh weight to 0.0065 mg/g fresh weight.

The possible environmental causation of anthocyanin concentration changes within each taxon was determined by the linear regression analysis. The results listed in Table 2 show that temperature significantly affects anthocyanin concentrations whereas moisture resulted in no effect.

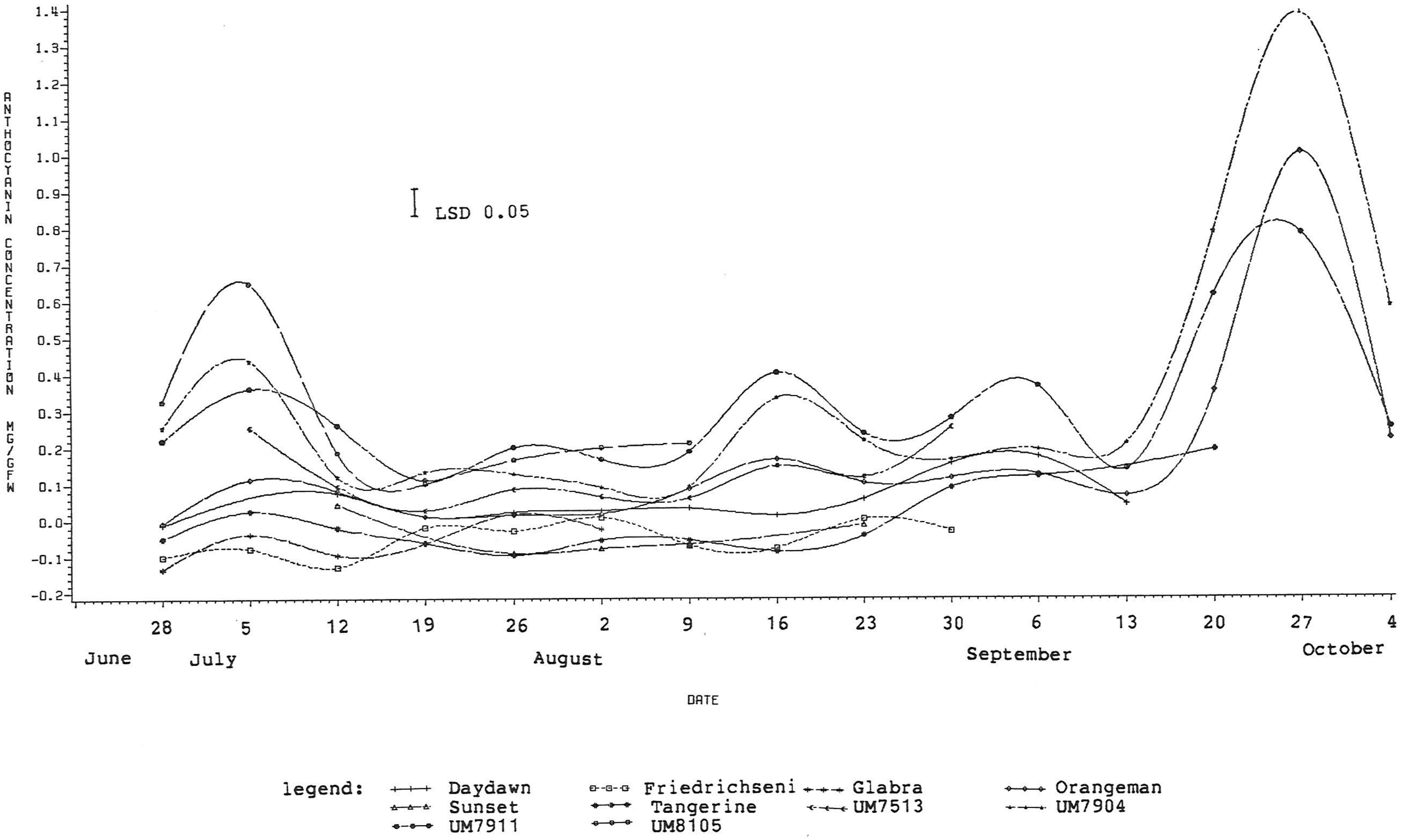


Figure 8: The mean anthocyanin concentrations of 10 taxa grown under field condtions

TABLE 2

The effect of air and soil temperatures and moisture on anthocyanin concentrations of plants grown under field conditions

	AIR TEMPERATURE						SOIL TEMPERATURE						MOISTURE	
TAXA	MAX7	MIN7	AV7	MAX14	MIN14	AV14	MAX7	MIN7	AV7	MAX14	MIN14	AV14	PRE	PSI
Acyanic														
Friedrichseni	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Glabra	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cyanic														
Daydawn	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Orangeman	ns	**	*	*	*	*	**	**	**	**	**	**	ns	ns
Sunset	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*	*	ns	ns
Tangerine	ns	ns	*	ns	*	*	**	**	**	**	*	**	ns	ns
UM7904	*	**	**	**	**	**	**	**	**	**	**	**	ns	ns
UM7911	ns	*	ns	*	ns	ns	*	*	*	*	*	*	ns	ns
UM7513	ns	ns	ns	ns	ns	ns	*	*	*	*	*	*	ns	ns
UM8105	**	ns	*	*	ns	*	**	*	**	ns	ns	ns	ns	ns

MAX7 :7day running maximum temperature (°C)
MIN7 :7day running minimum temperature (°C)
AV7 :7day running average temperature (°C)
MAX14:14day running maximum temperature (°C)
MIN14:14day running minimum temperature (°C)
AV14 :14day running average temperature (°C)
PRE :weekly precipitation (mm)
PSI :plant water potential (atms)

ns : not significant as determined by linear regression analysis
* : significant as determined by linear regression analysis (0.05)
** : significant as determined by linear regression analysis (0.01)

There was no observed significant effect of temperature on anthocyanin concentrations of acyanic plants, Glabra and Friedrichseni. The changes in concentrations during the season were the smallest and these values are within LSD(0.05).

Although there was a variation in the degree of significance among the taxa examined, the soil temperature effects were more pronounced than the air temperature effects. Among the various running temperatures, no one was any more significant than the others.

The values of slopes of significant regressions were all negative (Table 3) indicating that the increase in temperature resulted in a decrease in anthocyanin concentration.

Among the cyanic plants, only Daydawn was not affected by the temperature. UM7904 contained the highest concentration of pigment (1.38 mg/g fresh weight) among the taxa examined and was the most temperature sensitive. This is supported by the magnitude of the specimen slopes and the significance of the temperature data (Tables 2 and 3).

UM8105 contained the highest anthocyanin concentration at the beginning of July, however flowering ceased within a month. The flowers appeared reddish-orange, visually darker in "redness" than UM7904. It may have contained a much higher anthocyanin concentration in the fall. The plant vigor was poor.

TABLE 3

The slopes of significant linear regressions between air and soil temperatures and moisture, and anthocyanin concentrations

	AIR TEMPERATURE						SOIL TEMPERATURE						MOISTURE	
TAXA	MAX7	MIN7	AV7	MAX14	MIN14	AV14	MAX7	MIN7	AV7	MAX14	MIN14	AV14	PRE	PSI
Acyanic														
Friedrichseni														
Glabra														
Cyanic														
Daydawn														
Orangeman		-0.041	-0.032	-0.036	-0.046	-0.041	-0.042	-0.050	-0.046	-0.050	-0.059	-0.054		
Sunset										-0.032	-0.043	-0.037		
Tangerine							-0.020	-0.022	-0.021	-0.022	-0.023	-0.023		
UM7904	-0.036	-0.058	-0.047	-0.052	-0.060	-0.057	-0.054	-0.069	-0.061	-0.062	-0.074	-0.067		
UM7911		-0.021		-0.019			-0.021	-0.027	-0.024	-0.024	-0.028	-0.026		
UM7513			-0.013		-0.018	-0.015	-0.030	-0.040	-0.034	-0.034	-0.038	-0.036		
UM8105	-0.042		-0.042				-0.075	-0.079	-0.079					

MAX7 :7day running maximum temperature (°C)
MIN7 :7day running minimum temperature (°C)
AV7 :7day running average temperature (°C)
MAX14:14day running maximum temperature (°C)
MIN14:14day running minimum temperature (°C)
AV14 :14day running average temperature (°C)
PRE :weekly precipitation (mm)
PSI :plant water potential (atms)

A cyanic plant, Daydawn, was not affected by temperature and some cyanic plants were more colour stable than others, thus it may be possible to breed and select colour stable lines. However, it was observed that the more cyanic the flowers, the greater the temperature effect and the lesser the plant vigor. It is speculated that there may be a linkage between genes responsible for "red" colour (anthocyanin synthesis) and genes for poor plant vigor, and that anthocyanin synthesis in P. fruticosa is not consistent.

4.1.2.4 Carotenoids

Carotenoid concentration varied from 0.028 mg to 0.661 mg/g fresh weight (Figure 9). The carotenoid concentrations were lower than anthocyanin concentrations, but the visual colour of carotenoids is greater since the extinction coefficient is four times greater. The changes of the pigment concentration within a cultivar ranged from 85.9 percent to 30.8 percent and 0.359 mg/g fresh weight to 0.056 mg/g fresh weight.

According to linear regression analysis for carotenoid concentration change (Table 4), soil temperatures had a greater effect than the air temperatures. There was no detectable moisture effect on carotenoid concentrations except for Daydawn in which plant water potential resulted in a significant effect.

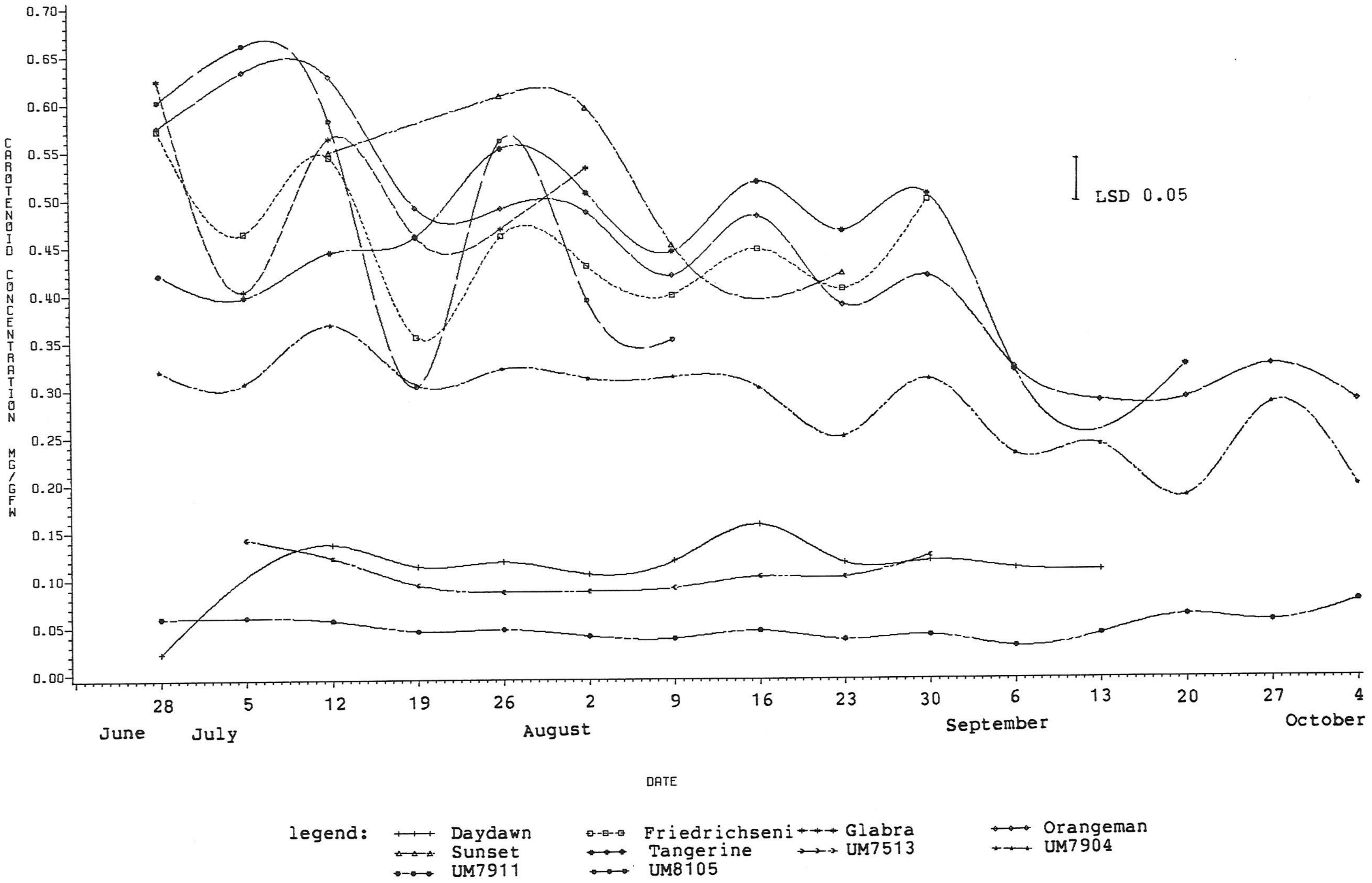


Figure 9: The mean carotenoid concentrations of 10 taxa grown under field conditions

TABLE 4

The effect of air and soil temperatures and moisture on carotenoid concentrations of plants grown under field conditions

	AIR TEMPERATURE						SOIL TEMPERATURE						MOISTURE	
TAXA	MAX7	MIN7	AV7	MAX14	MIN14	AV14	MAX7	MIN7	AV7	MAX14	MIN14	AV14	PRE	PSI
Acyanic														
Friedrichseni	ns	ns	ns	*	ns	*	ns	ns	ns	*	*	*	ns	ns
Glabra	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cyanic														
Daydawn	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	**
Orangeman	ns	ns	ns	ns	ns	ns	ns	*	ns	*	ns	ns	ns	ns
Sunset	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tangerine	*	ns	*	ns	ns	*	**	*	**	**	*	*	ns	ns
UM7904	**	*	**	ns	*	*	**	**	**	*	*	*	ns	ns
UM7911	**	*	**	**	**	**	*	*	*	**	**	**	ns	ns
UM7513	ns	ns	ns	*	*	*	*	**	*	**	**	**	ns	ns
UM8105	ns	ns	*	*	*	*	*	*	*	*	*	*	ns	ns

MAX7 :7day running maximum temperature (°C)
MIN7 :7day running minimum temperature (°C)
AV7 :7day running average temperature (°C)
MAX14:14day running maximum temperature (°C)
MIN14:14day running minimum temperature (°C)
AV14 :14day running average temperature (°C)
PRE :weekly precipitation (mm)
PSI :plant water potential (atms)

ns : not significant as determined by linear regression analysis
* : significant as determined by linear regression analysis (0.05)
** : significant as determined by linear regression analysis (0.01)

There was no significant effect of environmental parameters on the pigment concentrations of Glabra and Sunset. For both cultivars, there was about a 0.2 mg/g fresh weight in pigment concentration change, however the sample size of six sample days may have been insufficient for the analysis. The pigment concentrations of Daydawn, Friedrichseni, and Orangeman were significantly affected by temperatures. Tangerine, UM7904, UM7911, UM7513 and UM8105 were greatly temperature affected since there was higher linear regression significance between concentrations of these taxa and temperature. UM7904 and UM7911 were the two selection lines most affected by temperature. Although there was a similar percentage decrease in the concentrations (50 to 60 percent), the amount decreased differed considerably; 0.184 mg/g fresh weight for UM7904 and 0.046 mg/g fresh weight for UM7911.

The values of slopes of significant regressions varied among taxa (Table 5). The values for Orangeman, Tangerine and UM7904 were positive, concentration increased with an increase in temperature, whereas they were negative for Friedrichseni, UM7911, UM7513 and UM8105, indicating an increase in temperature resulted in decreased concentrations.

The considerable changes in the carotenoid concentrations observed in this experiment were unexpected since visually the yellow colours did not appear to change.

TABLE 5

The slopes of significant linear regressions between air and soil temperatures and moisture, and carotenoid concentrations

	AIR TEMPERATURE						SOIL TEMPERATURE						MOISTURE	
TAXA	MAX7	MIN7	AV7	MAX14	MIN14	AV14	MAX7	MIN7	AV7	MAX14	MIN14	AV14	PRE	PSI
Acyanic														
Friedrichseni				-0.014		-0.016				-0.024	-0.026	-0.025		
Glabra														
Cyanic														
Daydawn											0.011			
Orangeman								0.015		0.015				
Sunset														
Tangerine	0.009		0.010			0.012	0.015	0.017	0.016	0.017	0.019	0.018		
UM7904	0.006	0.007	0.006		0.007	0.006	0.008	0.008	0.008	0.007	0.007	0.007		
UM7911	-0.001	-0.002	-0.002	-0.002	-0.002	-0.002	-0.001	-0.002	-0.002	-0.002	-0.002			
UM7513				-0.006	-0.006	-0.006	-0.007	-0.010	-0.008	-0.011	-0.013	-0.012		
UM8105			-0.031	-0.037	-0.040	-0.039	-0.046	-0.058	-0.053	-0.046	-0.051	-0.048		

MAX7 :7day running maximum temperature (°C)
MIN7 :7day running minimum temperature (°C)
AV7 :7day running average temperature (°C)
MAX14:14day running maximum temperature (°C)
MIN14:14day running minimum temperature (°C)
AV14 :14day running average temperature (°C)
PRE :weekly precipitation (mm)
PSI :plant water potential (atms)

The difference in the effect of temperature on carotenoid concentration among taxa indicates that not only can a temperature stable line be selected but also a line in which pigment concentration would increase with temperature may be produced. A similar result was reported for tomato fruit, in which the temperature effect varied on each type of carotenoid involved depending upon genotype (Goodwin, 1966). Quantitative analysis of each carotenoid present in _P. fruticosa_ would clarify this point.

The effect of the short term, 7 day, and the long term, 14 day, running temperature on carotenoid concentration did not differ. A possible difference may have been negated due to the variability in the flower development rate, which appeared to be temperature dependent.

Unlike the anthocyanins, the plants with higher carotenoid concentrations are not necessarily more affected by temperature. Some of the more temperature affected taxa contained very low carotenoid concentrations; UM7911 and UM7513. Although the coefficients were highly significant, the slopes were very low, -0.001 to -0.002 and -0.006 to -0.013, respectively.

4.1.3 Temperature Effects

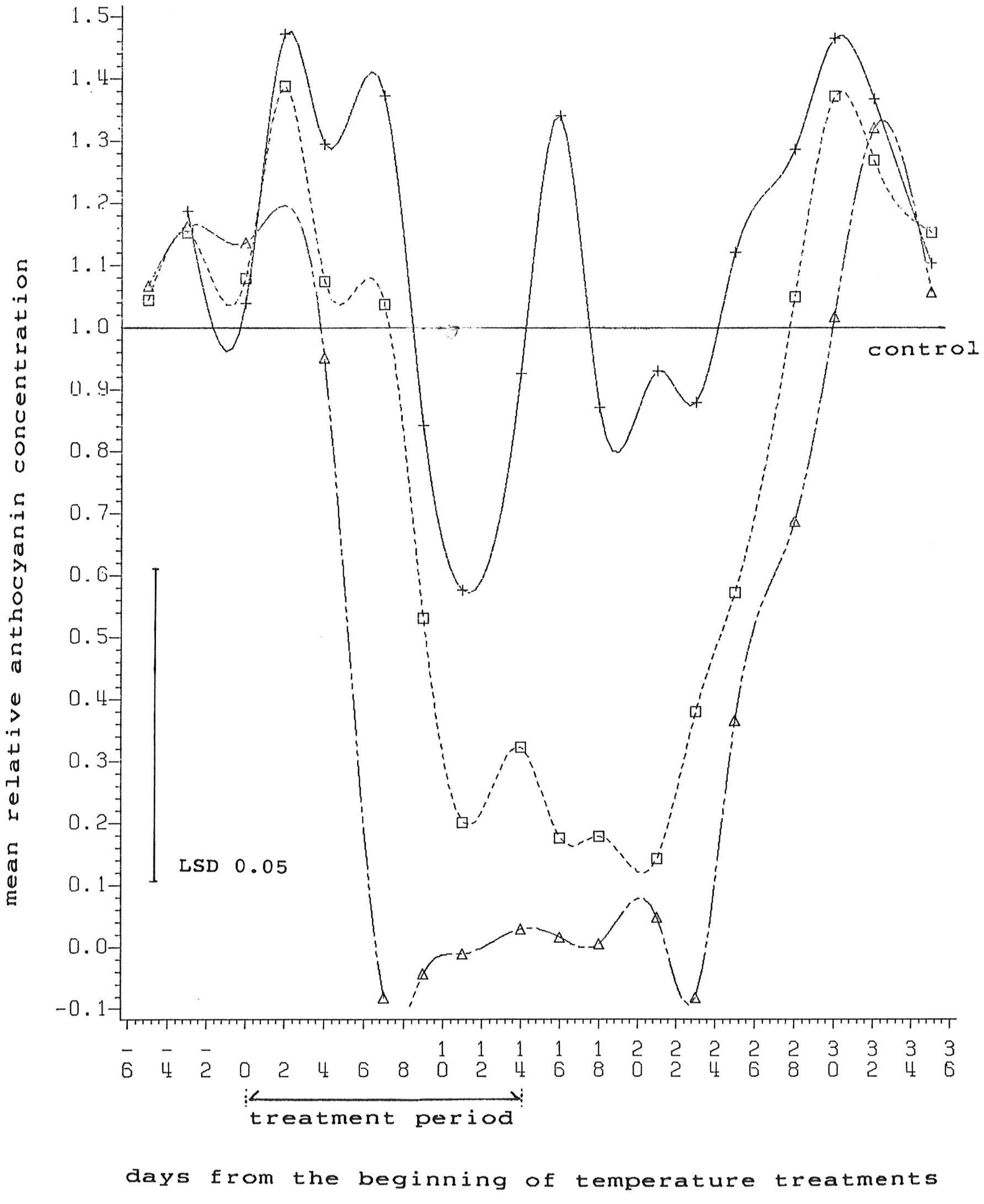
Under controlled environment, flower size decreased with higher temperature treatments. Vegetative growth as well as the rate of flower development increased with an increase in temperature. The flower colour change from cyanic to acyanic occurred visually. Of the three times the treatments were repeated, the first set of treatment data was not used due to considerable plant damage and missing values.

4.1.3.1 Anthocyanin

Anthocyanin concentration changed within the control (15/5°C) throughout the experiment, although visually this was difficult to recognize. Since the three temperature treatments were not applied simultaneously, pigment concentrations were expressed as a ratio to the control (Figures 10 and 11).

The anthocyanin concentration decreased with an increase in temperature in all treatments. The reduction in concentration was greater for UM7904 than UM7911 and the highest temperature treatment (30/15°C) reduced the concentration to zero.

For UM7904 there was a significant difference in concentrations among treatments. One initial increase at day two in all treatments was attributed to a decrease in petal fresh weight and not an increase in total pigment weight. The largest decrease occured at the 30/20°C temperature treatment and a plateau was reached within a week. The rate of concentration decrease was smallest for the 20/10°C treatment.



legend: ┼┼┼ 20/10°C ⊡-⊡-⊡ 25/15°C △-△-△ 30/20°C

Figure 10: The effect of temperature treatments on anthocyanin concentration of UM7904 grown in controlled environment

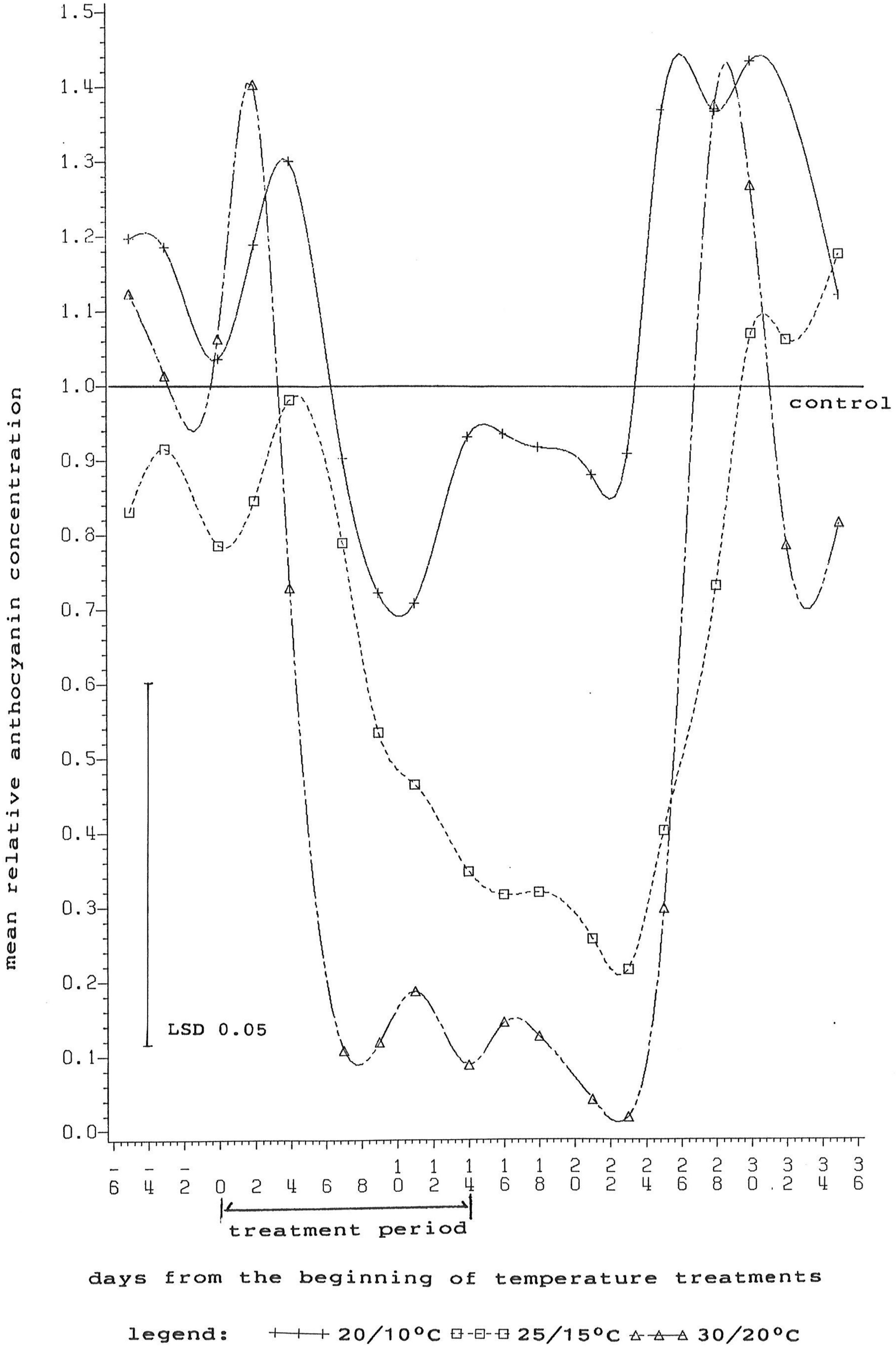


Figure 11: The effect of temperature treatments on anthocyanin concentration of UM7911 grown in controlled environment

After the removal from the treatment temperatures (30/20°C, 25/15°C, and 20/10°C), to the control temperature (15/5°C), concentrations increased towards the level of control. Recovery was most rapid for the 20/10°C treatment group, whereas over two weeks was required to return to the original level for the 30/20°C treatment group.

A similar trend was observed for UM7911. The effect of the temperature treatment differed significantly among treatments and the percentage concentration decrease was less than that for UM7904, and the concentration decrease was smaller.

The change of flower colour from cyanic to acyanic is due to lack of anthocyanin synthesis under high temperatures. The pigments already present in the tissue did not disappear due to the high temperature treatment, thus the initial concentration increase observed on day two was attributed to a decrease in fresh weight. The decreased pigment synthesis was apparent by day 7. At 30/20°C the most rapid response may be due to either the strongest effect of temperature on the anthocyanin synthesis or the most rapid appearance of the plant response due to more rapid flower development induced by high temperature.

4.1.3.2 Carotenoids

The effect of temperature differed between the two selection lines. The selection line UM7901 showed an increase in pigment concentration at each of the temperatures but the differences were not significant. However there was a significant difference between days (Figure 12).

The concentration decreased with an increase in temperature for UM7911 and the differences were not significant. The highest temperature treatment, 30/20°C, resulted in the greatest reduction in the concentration, although a small amount of pigment was still present (Figure 13).

The result observed here that the temperature treatments resulted in opposing effects supports the earlier findings from the field experiment; the regression coefficients were positive for UM7904 and negative for UM7911.

Although the carotenoid concentration changed, the visual effect was not pronounced. The greater carotenoid concentration in UM7904 probably enhances the visual disappearance of anthocyanin, whereas the carotenoid concentration decrease in UM7911 results in a greater visual effect of anthocyanins.

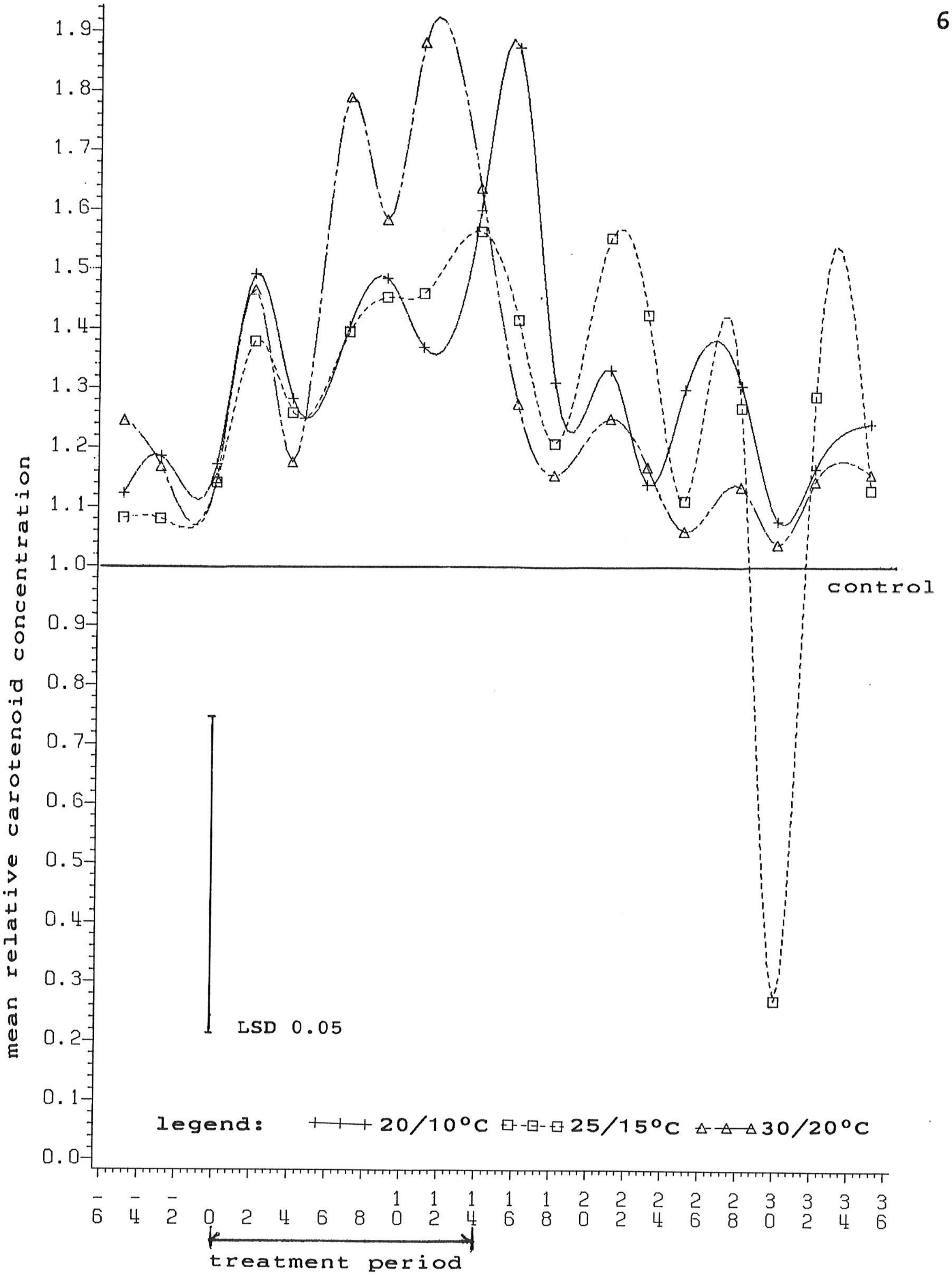


Figure 12: The effect of temperature treatments on carotenoid concentration of UM7904 grown in controlled environment

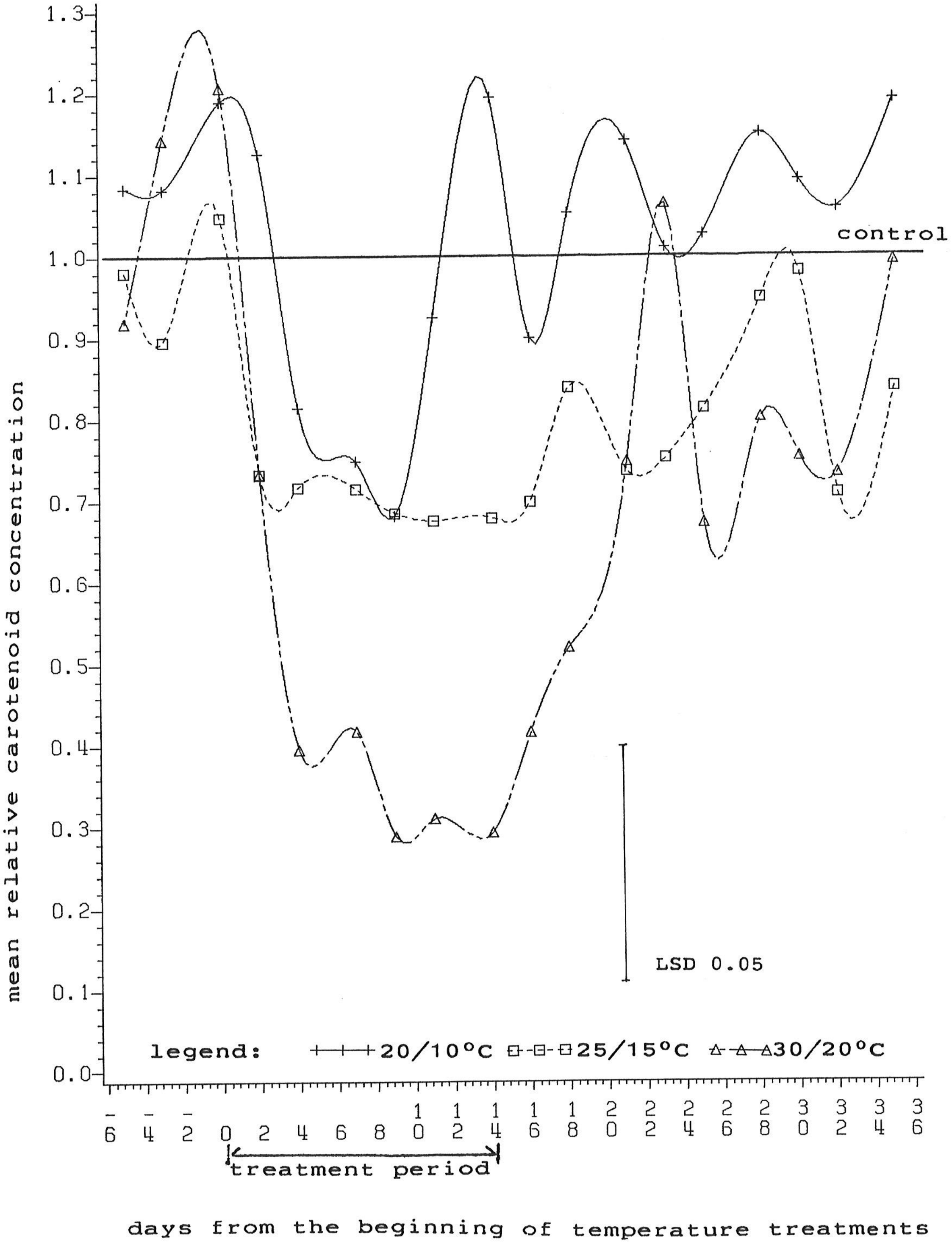


Figure 13: The effect of temperature treatments on carotenoid concentration of UM7911 grown in controlled environment

4.1.4 Flower Bud Development and Pigment Synthesis

4.1.4.1 Bud Development

Bud diameter increased the most at the time of flower opening (between stages 5 and 6), whereas the petal fresh weight increased rapidly between stages 2 and 3 and between stages 5 and 6 (Figures 14 and 15).

There was some effect of higher temperature (30/20°C) on bud growth . The bud diameter was reduced, most obvious when the flowers were open (stages 6 to 8), a reduction of about 35 percent at anthesis (stage 6). There was a greater reduction in petal weight, about 65 percent decrease at anthesis (stage 6). The petal weight reduction is mainly due to the loss of two growth periods (between stages 2 and 3 and between stages 5 and 6) when there was rapid increase in fresh weight at 15/5°C.

4.1.4.2 Anthocyanins

At 15/5°C, the anthocyanin concentration was greatest at stage 2 and there was a gradual decrease thereafter with a slight increase during the senescence (Figure 16). The total anthocyanin increased throughout flower development and most of the increase occurred during the early stages of bud development; about 50 percent of the synthesis occurred from stage 1 to stage 2 (Figure 17). A high temperature treatment at about this time is expected to have the greatest effect on the decrease in pigment synthesis. At

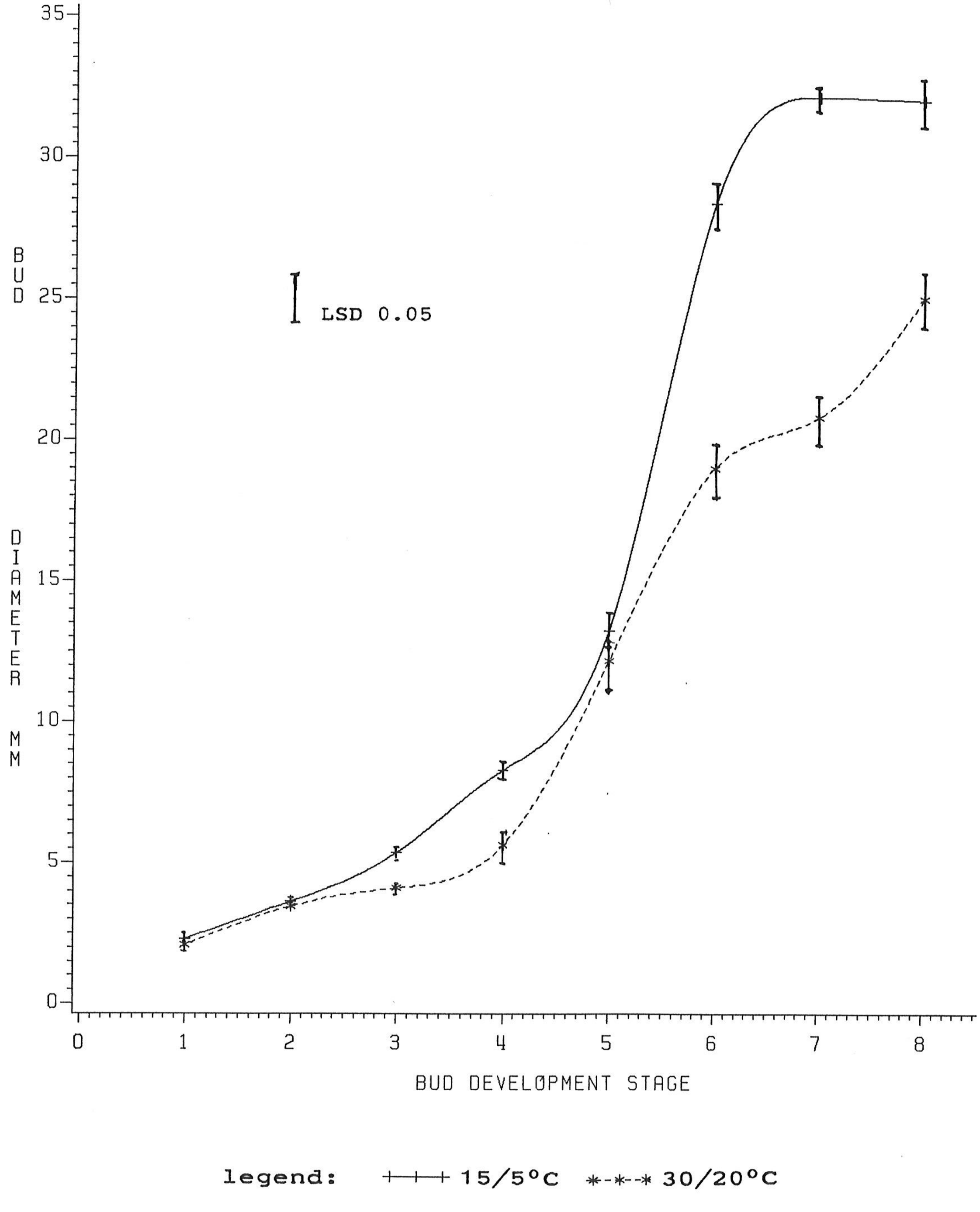


Figure 14: The mean bud diameter of UM7904 during flower development on plants grown in controlled environment

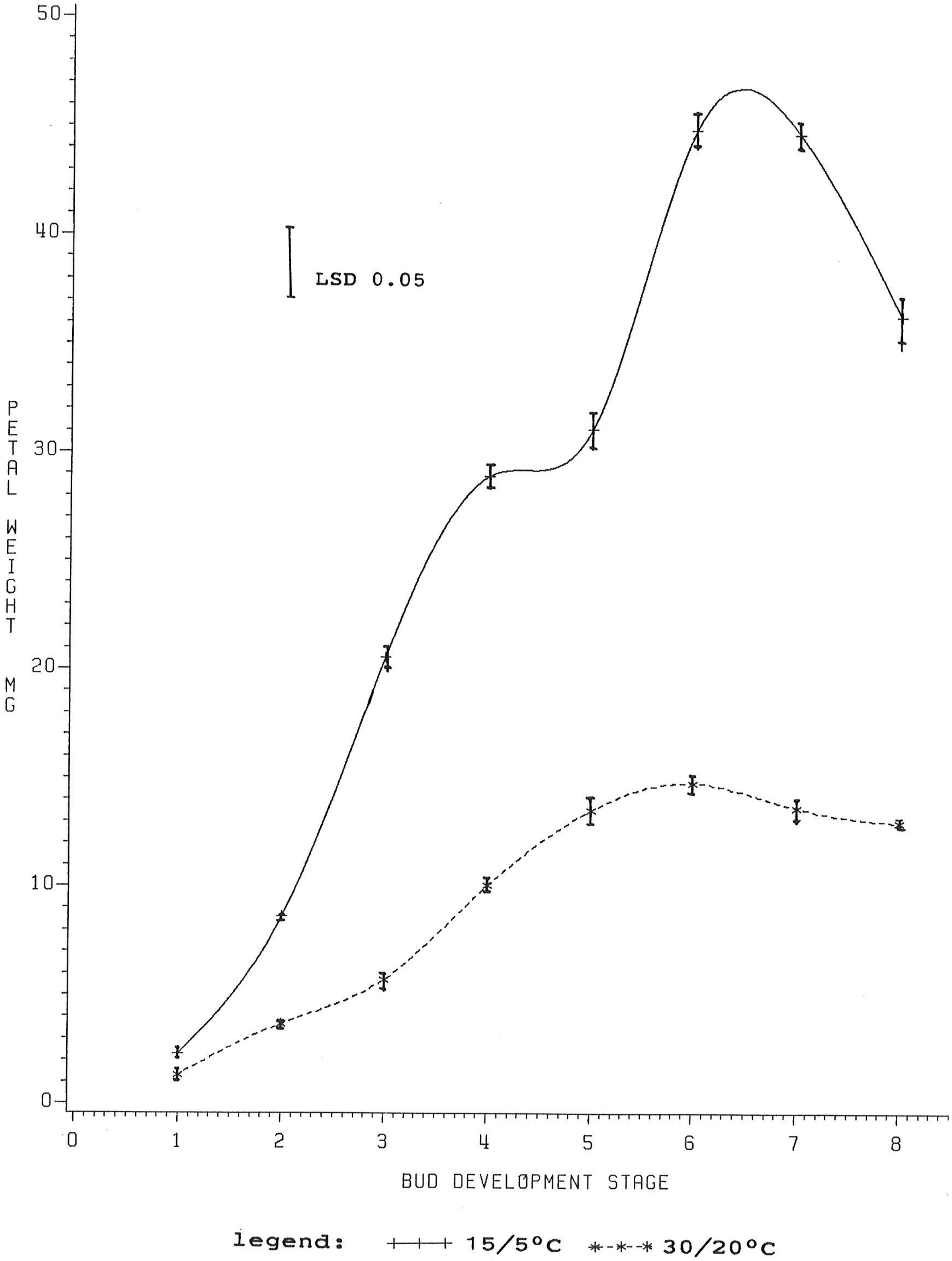


Figure 15: The mean petal weight of UM7904 during flower development on plants grown in controlled environment

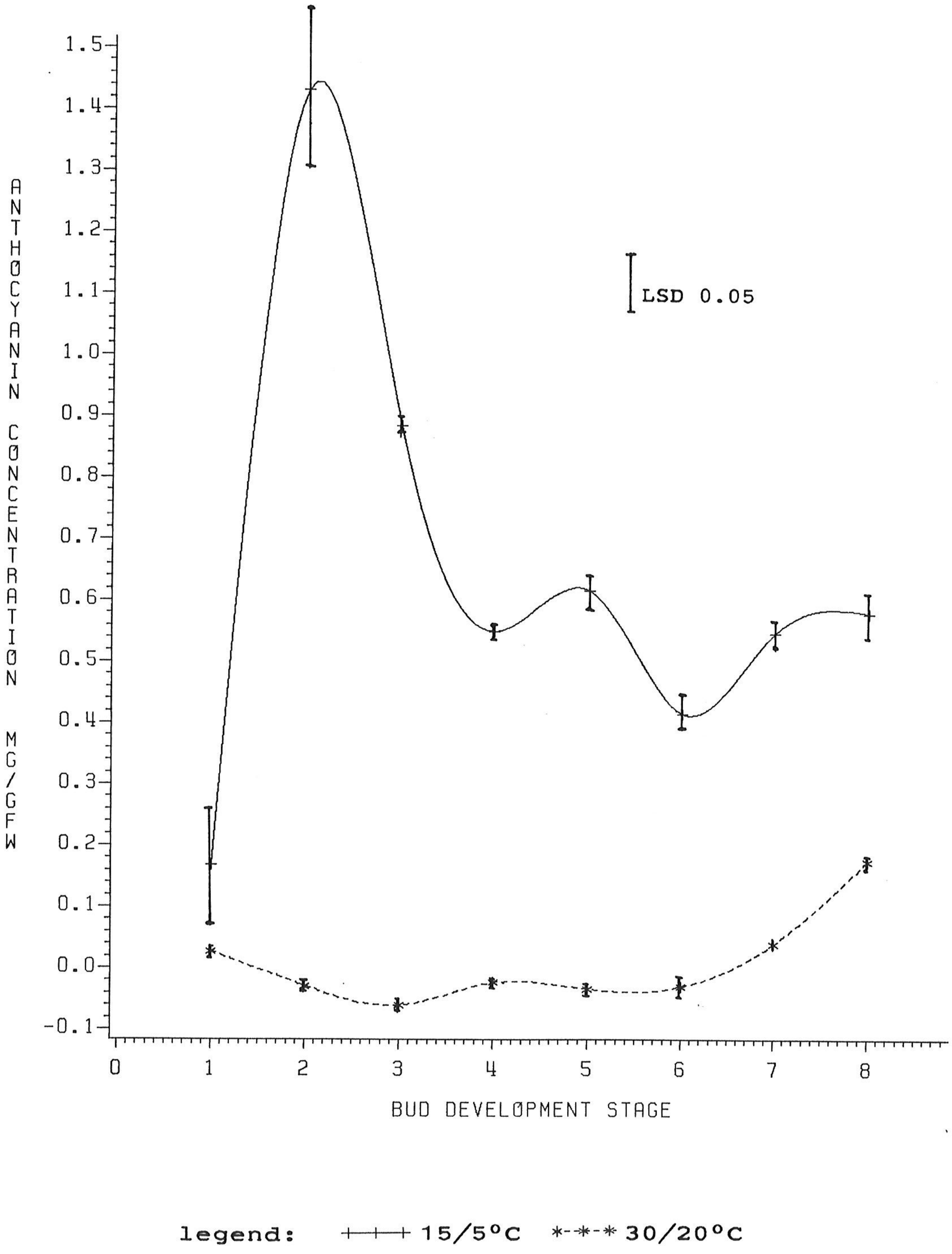


Figure 16: The mean petal anthocyanin concentration of UM7904 during flower development on plants grown in controlled environment

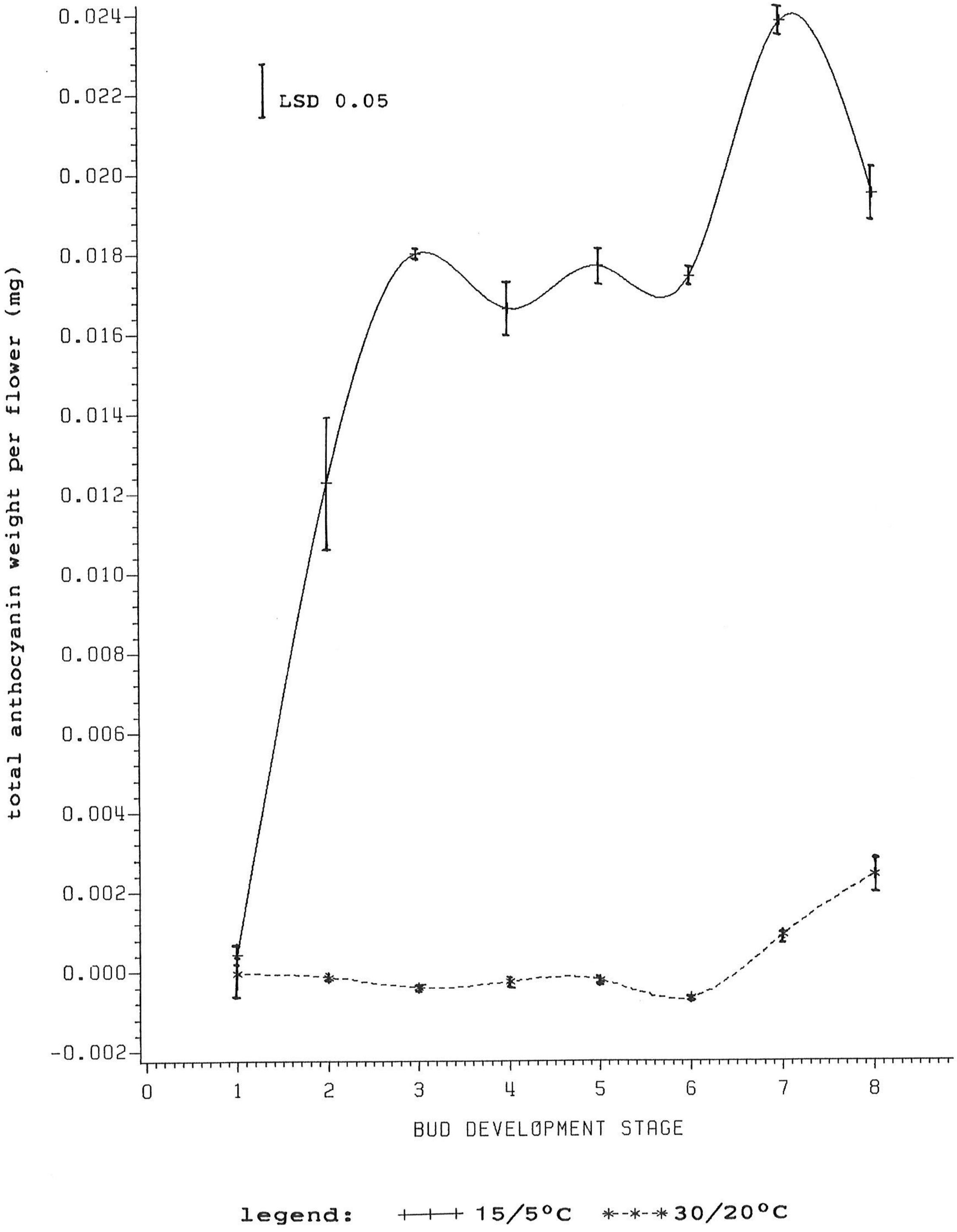


Figure 17: The mean total petal anthocyanin content of UM7904 during flower development on plants grown in controlled environment

higher temperature, 30/20°C, both the concentration and the total anthocyanin remained near zero. The presence of pigment at stages 7 and 8 is not due to pigment synthesis at this time, but probably to a residual effect of earlier synthesis when these buds were initiated at 15/5°C (control), since these plants were sampled at the end of a treatment period of two weeks.

The anthocyanin concentration reduction after stage 2 is due to dilution from the rapid fresh weight increase since the total pigment weight increased throughout development.

Light was not directly required for anthocyanin synthesis. The flower buds were enclosed in bracts until stage 3 and the greatest pigment synthesis occurred between stages 1 and 2. It is possible that the bracts act as a mediator in the light receptor of anthocyanin synthesis.

The independence of anthocyanin synthesis from light is further observed in that the anthocyanin occurred only in the upper epidermis of petals, which was not exposed to light until stage 6, and by this time most, if not all, of the pigments were already present. This observation is in contrast with the findings of Armitage and Carlson (1981) on marigold leaves, Biran and Halevy (1974a and 1974b) on Baccara rose flowers, and Stickland (1972) on <u>Chrysanthemum morifolium</u> in which there was a strong dependence of anthocyanin synthesis on light.

4.1.4.3 Carotenoids

At 15/5°C, the maximum carotenoid concentration occurred at stage three and there was a gradual decrease until senescence (stage 8) (Figure 18). The maximum total carotenoid occurred at anthesis (stage 6) and there was a rapid increase from stage 2 to stage 3 (Figure 19).

At the higher temperature of 30/20°C, the carotenoid concentration was slightly lower than at 15/5°C until stage 6, however the total carotenoid was much less, about one third of that at 15/5°C, reaching the maximum at anthesis (stage 6). The small difference in carotenoid concentrations at two temperatures, despite the much lower total pigment synthesis at 30/20°C, was accounted for by the much reduced fresh weight. The increase in carotenoid concentration at the higher temperature after stage six cannot be explained. The higher concentration at anthesis is in accordance with the earlier findings of the controlled environment experiment. Light appears to be not directly required for carotenoid synthesis.

The lower total carotenoid at 30/20°C is mainly attributed to the noticeable reduction in pigment synthesis between stages 2 and 3. The greatest effect of high temperature is expected to occur at this time.

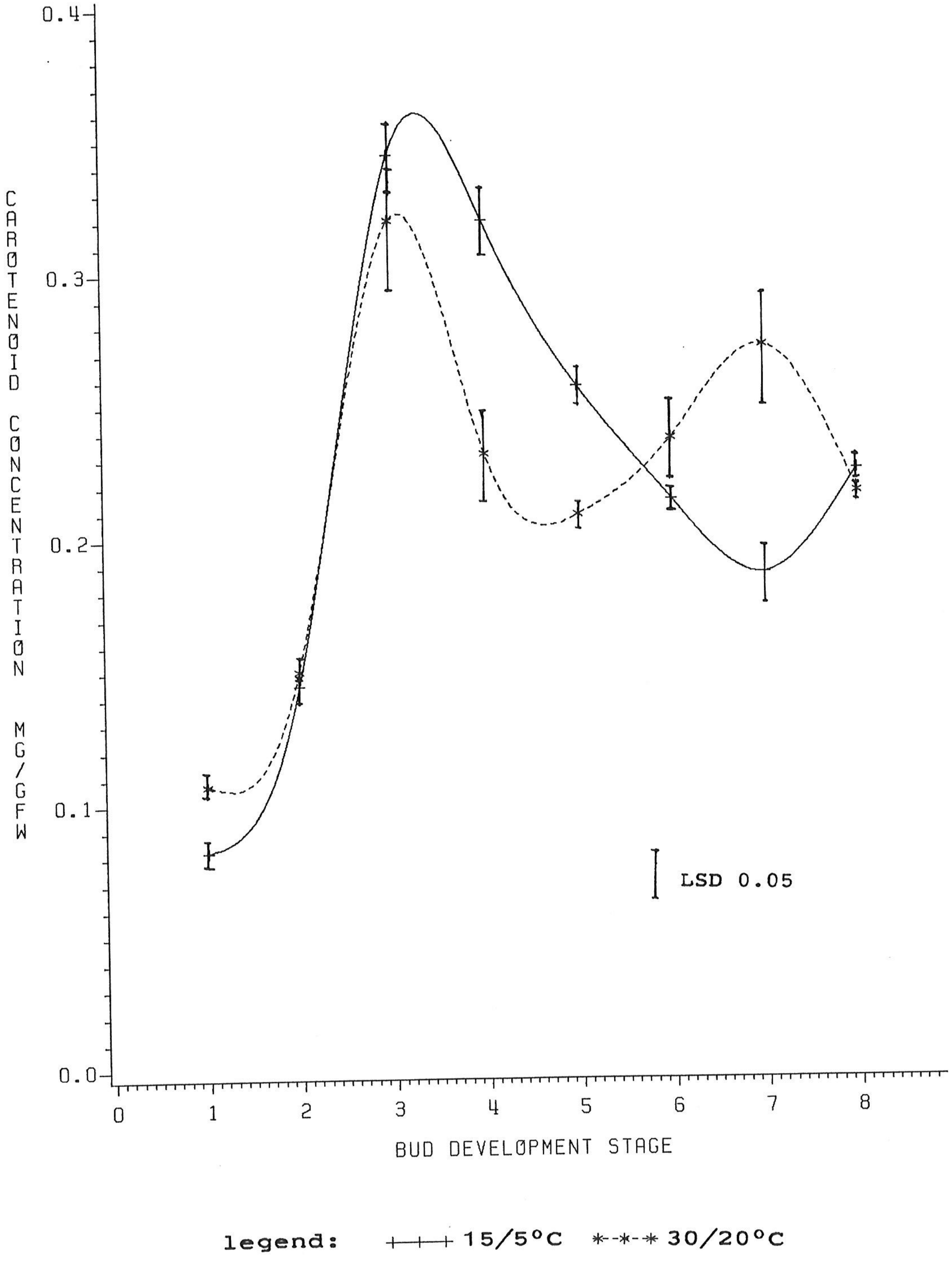


Figure 18: The mean petal carotenoid concentration of UM7904 during flower development on plants grown in controlled environment

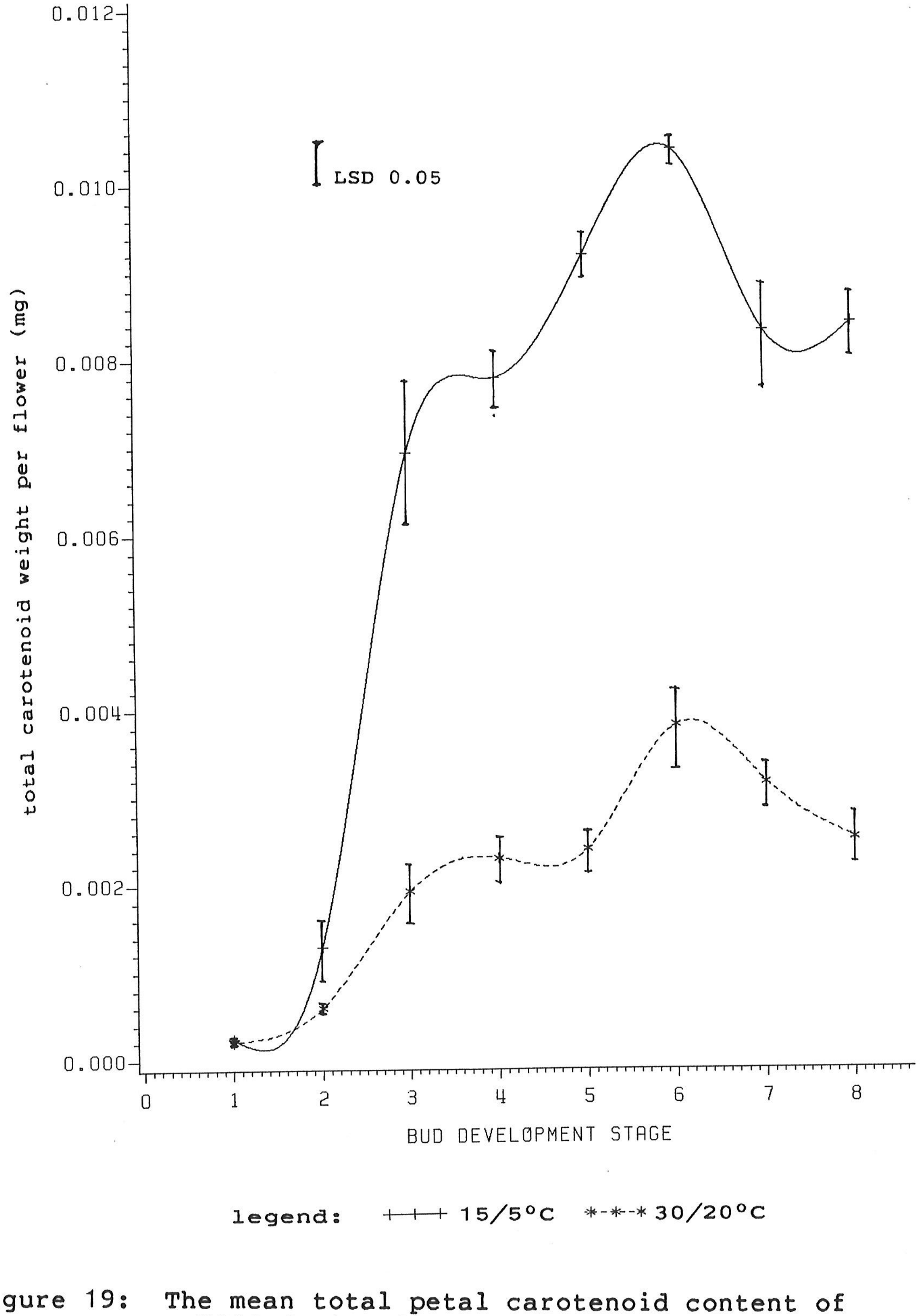


Figure 19: The mean total petal carotenoid content of UM7904 during flower development on plants grown in controlled environment

4.2 PETAL NUMBER

4.2.1 Environmental Effects

4.2.1.1 General Evaluation

There were 25 extra-petalled taxa and 136 five-petalled taxa (Appendix A). All the extra-petalled taxa were selection lines except Hersi and Sundance. The cyanic taxa usually did not show any extra petals under field conditions. The mean duration of the flowering period for the extra-petalled taxa was 99.0 days and that for the five-petalled taxa was 91.2 days (Table 6). The means differed significantly by Duncan's test (0.05).

4.2.1.2 Petal Number Change

Petal number ranged from five to 15 and there appears to be a gene block at 15 petals (Figure 20). The change in the petal number varied among taxa; the petal number of Hersi remained relatively low throughout the season, those of Sundance and UM7901 increased gradually towards the end of the season, and that of UM8102 increased rapidly at the beginning of the season and remained relatively high for most of the season.

The linear regression analysis was carried out to determine the environmental cause of petal number change (Table 7). The petal number was more affected by the longer, 14 day running temperatures, than the shorter, 7 day running

TABLE 6

The mean flower period by petal number groups

PETAL NUMBER	FLOWER PERIOD (DAYS)*
more than 5	99.00 a
5	91.23 b

*Flower period values with the same letter did not differ significantly as determined by the Duncan's test (0.05)

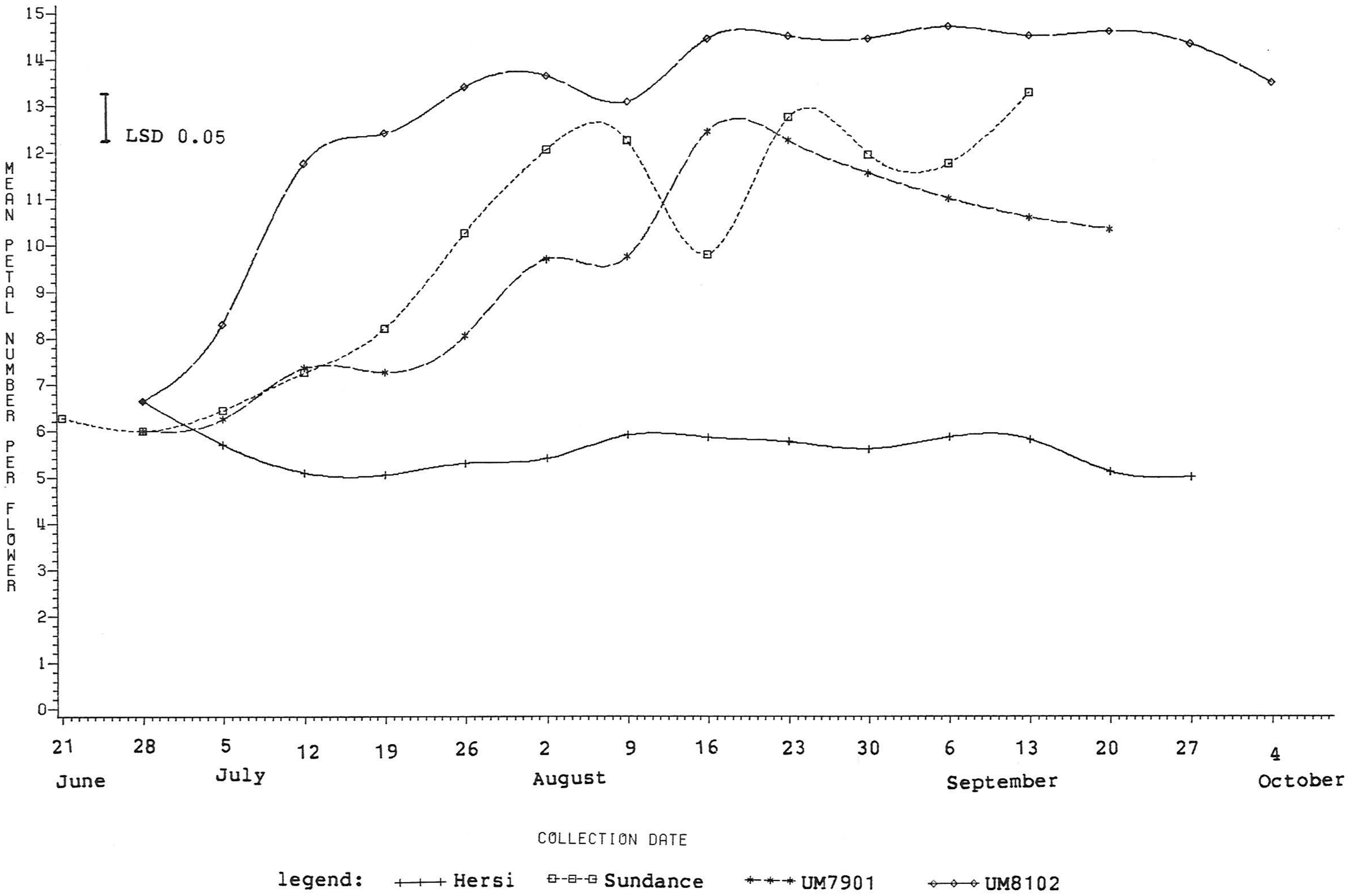


Figure 20: The mean petal number of four taxa grown under field conditions

legend to tables 7 and 8

MAX7-0	7day running maximum temperature to the day of sampling (°C)
MIN7-0	7day running minimum temperature to the day of sampling (°C)
AV7-0	7day running average temperature to the day of sampling (°C)
MAX7-1	7day running maximum temperature one week before sampling (°C)
MIN7-1	7day running minimum temperature one week before sampling (°C)
AV7-1	7day running average temperature one week before sampling (°C)
MAX7-2	7day running maximum temperature two weeks before sampling (°C)
MIN7-2	7day running minimum temperature two weeks before sampling (°C)
AV7-2	7day running average temperature two weeks before sampling (°C)
MAX14-0	14day running maximum temperature to the day of sampling (°C)
MIN14-0	14day running minimum temperature to the day of sampling (°C)
AV14-0	14day running average temperature to the day of sampling (°C)
MAX14-1	14day running maximum temperature one week before sampling (°C)
MIN14-1	14day running minimum temperature one week before sampling (°C)
AV14-1	14day running average temperature one week before sampling (°C)
MAX14-2	14day running maximum temperature two weeks before sampling (°C)
MIN14-2	14day running minimum temperature two weeks before sampling (°C)
AV14-2	14day running average temperature two weeks before sampling (°C)

PRE0	weekly precipitation for the week of sampling (mm)
PSI0	plant water potential for the day of sampling (atms)
PRE1	weekly precipitation one week before sampling (mm)
PSI1	plant water potential one week before sampling (atms)
PRE2	weekly precipitation two weeks before sampling (mm)
PSI2	plant water potential two weeks before sampling (atms)

TABLE 7

The effect of air and soil temperatures and moisture on petal number of plants grown under field conditions

Air Temperature

TAXA	MAX7-0	MIN7-0	AV7-0	MAX7-1	MIN7-1	AV7-1	MAX7-2	MIN7-2	AV7-2
Hersi	ns	ns	ns	ns	ns	ns	*	ns	*
Sundance	ns	ns	ns	*	ns	*	*	*	**
UM7901	ns	ns	ns	ns	ns	ns	**	**	**
UM8102	ns	ns	ns	ns	ns	ns	ns	ns	ns

TAXA	MAX14-0	MIN14-0	AV14-0	MAX14-1	MIN14-1	AV14-1	MAX14-2	MIN14-2	AV14-2
Hersi	ns	ns	ns	ns	ns	ns	*	ns	ns
Sundance	*	ns	ns	**	*	**	**	**	**
UM7901	ns	ns	ns	**	**	**	**	**	**
UM8102	ns	ns	ns	ns	ns	ns	ns	ns	ns

Soil Temperature

TAXA	MAX7-0	MIN7-0	AV7-0	MAX7-1	MIN7-1	AV7-1	MAX7-2	MIN7-2	AV7-2
Hersi	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sundance	ns	ns	ns	ns	*	*	*	**	**
UM7901	ns	ns	ns	ns	ns	ns	*	**	**
UM8102	ns	ns	ns	ns	ns	ns	ns	ns	ns

TAXA	MAX14-0	MIN14-0	AV14-0	MAX14-1	MIN14-1	AV14-1	MAX14-2	MIN14-2	AV14-2
Hersi	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sundance	ns	ns	ns	**	**	**	**	**	**
UM7901	ns	ns	ns	*	**	*	**	**	**
UM8102	ns	ns	ns	ns	ns	ns	ns	*	*

Moisture

TAXA	PRE0	PSI0	PRE1	PSI1	PRE2	PSI2
Hersi	ns	*	ns	ns	ns	ns
Sundance	ns	*	ns	ns	ns	*
UM7901	ns	*	ns	ns	ns	*
UM8102	ns	**	ns	*	ns	ns

ns : not significant as determined by linear regression analysis
* : significant at 0.05
** : significant at 0.01

temperatures. There was as much effect by air temperature as by soil temperature. The effect of the running temperatures to the day two weeks before the sampling was greater than that to one week before. There was no effect by running temperatures to the day of sampling. The temperature conditions up to the time of flower bud initiation or around the time of early bud development appears to be most important.

UM8102 was least affected by the temperature. The temperature effect on Hersi is uncertain since the cultivar changed little in petal number. Sundance and UM7901 were affected by temperature the most.

Precipitation did not affect petal number in all taxa. There were significant regression coefficients between plant water potentials and petal number for all four taxa. It was expected that the water potential would have affected the petal number at the time of flower initiation, if at all. However, the greatest effect on the petal numbers resulted from the water potential of the day of sampling, rather than that of one or two weeks before according to the linear regression analysis.

The slopes of significance for temperatures were positive (Table 8), indicating that petal number increased with increase in temperature. Those for plant water potentials were mostly negative. Petal number decreased

TABLE 8

The slopes of significant linear regressions between air and soil temperatures and moisture, and petal numbers

Air Temperature

TAXA	MAX7-0	MIN7-0	AV7-0	MAX7-1	MIN7-1	AV7-1	MAX7-2	MIN7-2	AV7-2
Hersi							0.05		0.06
Sundance				0.50		0.50	0.38	0.56	0.54
UM7901							0.40	0.54	0.55
UM8102									

TAXA	MAX14-0	MIN14-0	AV14-0	MAX14-1	MIN14-1	AV14-1	MAX14-2	MIN14-2	AV14-2
Hersi							0.06		
Sundance	0.56			0.75	0.68	0.76	0.66	0.66	0.69
UM7901				0.57	0.62	0.63	0.61	0.68	0.68
UM8102									

Soil Temperature

TAXA	MAX7-0	MIN7-0	AV7-0	MAX7-1	MIN7-1	AV7-1	MAX7-2	MIN7-2	AV7-2
Hersi									
Sundance					0.35	0.62	0.42	0.16	0.28
UM7901							0.52	0.80	0.65
UM8102									

TAXA	MAX14-0	MIN14-0	AV14-0	MAX14-1	MIN14-1	AV14-1	MAX14-2	MIN14-2	AV14-2
Hersi									
Sundance				0.17	0.08	0.12	0.15	0.13	0.14
UM7901				0.50	0.71	0.60	0.69	0.86	0.77
UM8102								0.35	0.31

Moisture

TAXA	PRE0	PSI0	PRE1	PSI1	PRE2	PSI2
Hersi		0.08				
Sundance		-0.47				-0.39
UM7901		-0.37				-0.34
UM8102		-0.48		-0.45		

with an increase in plant water stress. The linear regression slope for Hersi was positive, however the value was relatively small and considering that the cultivar had an overall petal number change of 1.6, it may not have been a suitable material for this study. There may be two types of petal number change within the species. One having a higher petal number at the beginning of the season would have less towards the end (Hersi), and the other would increase in petal number as the season progressed (Sundance, UM7901, and UM8102). Since these two groups are opposite in nature, the moisture effect may be only secondary. It appeared in the field that the plants with vigorous growth tended to produce more petals. It was reported that double flowers were associated with vigorous growth caused by such factors as cultivation in rich soil, later flowering, or young, vigorous trees (Reynolds and Tampion, 1983). The net photosynthesis or sugar availability may have had a greater effect than the moisture factor.

4.2.2 Temperature Effects

The petal number under controlled environment conditions varied over time for each taxon studied, however there was no significant effect of temperature treatments on the petal number on UM7901 and UM8102 (Figures 21 and 22).

A linear correlation analysis was carried out between petal number and flower number. Both selection lines showed

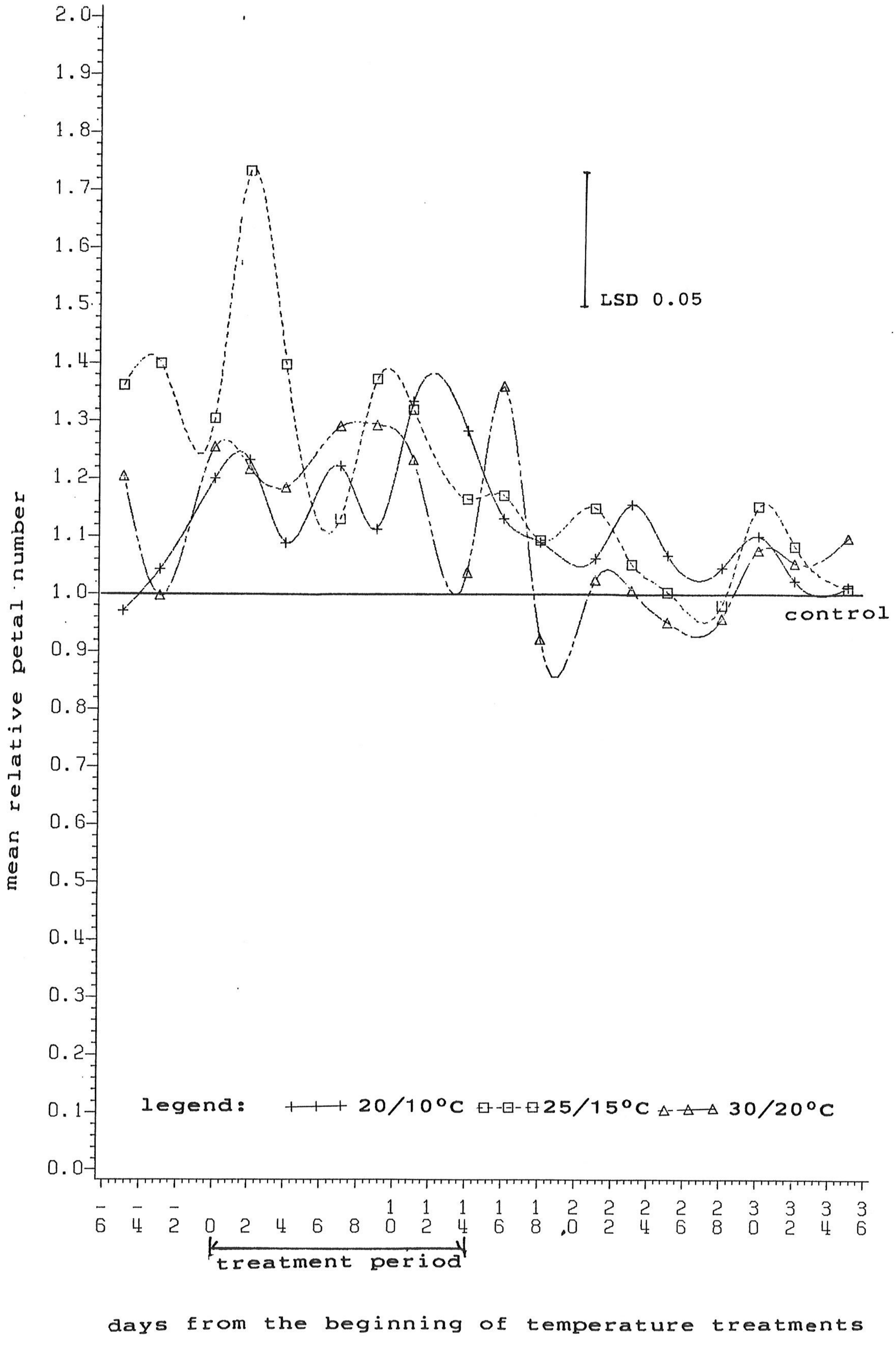


Figure 21: The effect of temperature treatments on the petal number of UM7901 grown in controlled environment

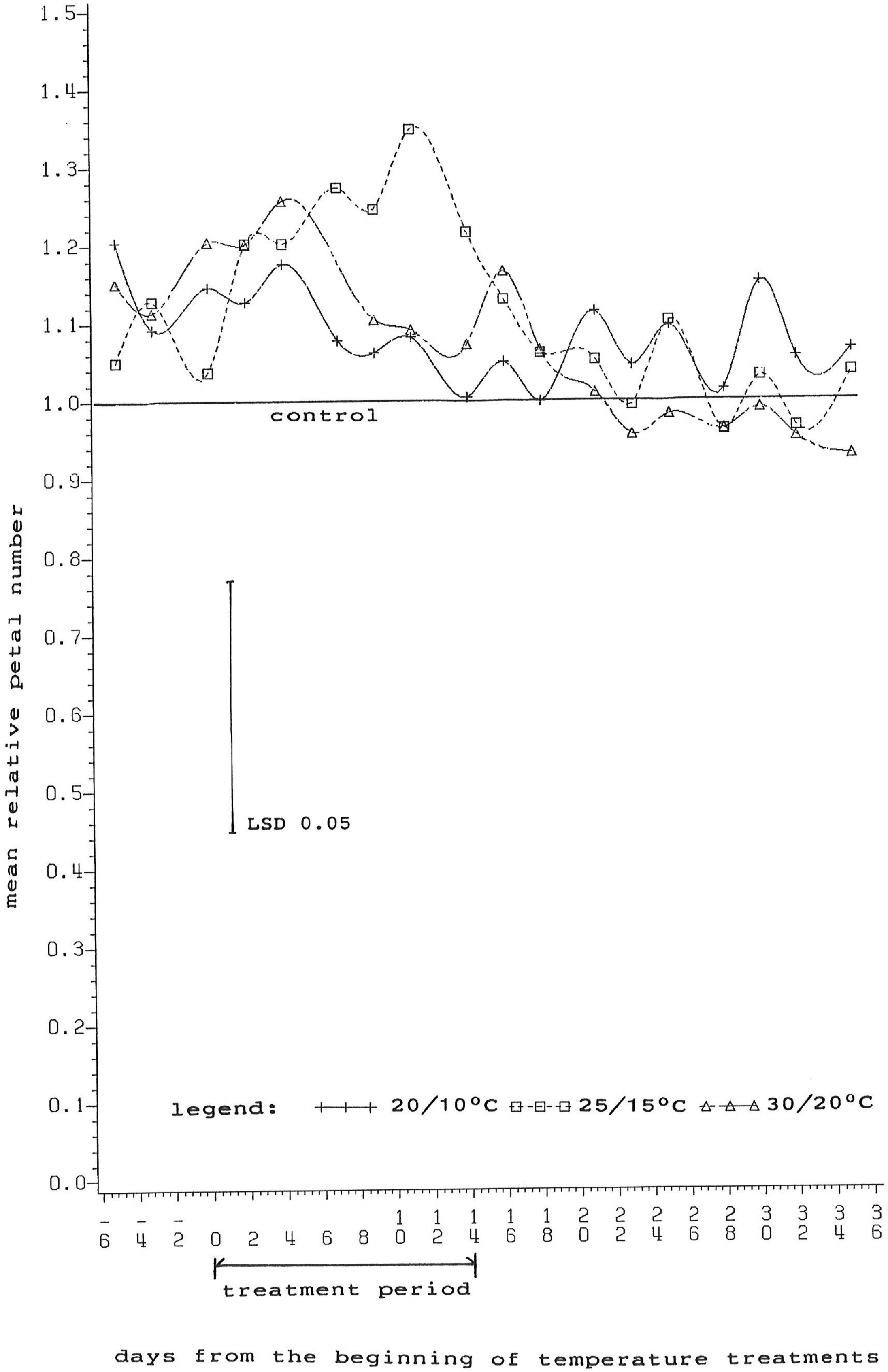


Figure 22: The effect of temperature treatments on the petal number of UM8102 grown in controlled environment

a very significant negative correlation between petal number and flower number per plant (Table 9). The petal number increase significantly correlated with flower number decrease.

Further experiment regarding plant moisture status was not conducted due to the foreseen difficulty of obtaining a steady moisture state over a period of more than two weeks.

TABLE 9

The relationship between petal numbers and flower numbers

selection line	n	r	slope	intercept
UM7901	307	-0.2363 **	-0.00367	8.8122
UM8102	304	-0.2202 **	-0.00404	11.264

** correlation was highly significant as determined by linear correlation analysis (0.0001)

Chapter V

GENERAL DISCUSSION

5.1 FLOWER COLOUR

Cyanic taxa of *P. fruticosa* derive their "redness" from the production of anthocyanins. The pigments are not usually found in wild forms, however many plants of the genus *Potentilla* and the family Rosaceae commonly contain this group of pigments (Harborne, 1967; Hrazdina, 1982) and the genetic potential was expected to be present in *P. fruticosa*. The first cyanic cultivar probably arose as a natural mutation.

Other plant pigments present in the flowers of *P. fruticosa* are carotenoids, which are responsible for the yellow colour and flavonoids other than anthocyanins which are responsible for white. The white colour may also be the result of light reflection on the petals (Stickland, 1974b).

The flower colour change of cyanic taxa from cyanic to acyanic during the growing season is mainly due to reduction of anthocyanin synthesis under higher temperatures. Temperature also affects carotenoid synthesis, however the effect on visual colour is much less noticeable.

Although anthocyanins are often synthesized in response to stress conditions (Hrazdina, 1982), this is unlikely for red colouration in P. fruticosa flowers.

The optimum temperature for maximum metabolic activities varies from species to species (Treshow, 1970). The range of temperature is closely correlated with the seasonal and daily temperature ranges of the native habitats (Turner and Kramer, 1980). P. Fruticosa, native to the circumpolar region in the northern hemisphere (Bailey and Bailey, 1976), is expected to have optimum temperature similar to temperature occurring within the regional growing temperature. Anthocyanins were produced only at the lower extreme of growing temperature and not at the higher extreme.

Anthocyanin synthesis may be explained by the effect of temperature on carbohydrate metabolism. There are many reports supporting this theory (Biran and Halevy, 1974a and 1974b; Creasy, 1968 and 1974; Rutland and Seawright, 1973).

The effect of sugars on anthocyanin formation was reported as early as 1949 by Thimann and Edmondson. Spirodela oligorrhiza in a modified Hoagland's solution showed a marked increase in anthocyanin formation with added sugars in the culture. Sucrose promoted anthocyanin formation, whereas glucose promoted growth (Thimann et al, 1951). There was a direct correlation between anthocyanin and reducing sugars in the tissue. Chrysanthemum morifolium cv. Fandango cultured florets also produced the highest amount of anthocyanin with four percent sucrose in the medium (Stickland, 1974a).

The net carbohydrate accumulation which could serve for pigment synthesis, is determined by the combined effects of photosynthesis, respiration, and growth. In general, optimum temperatures for photosynthesis are similar to the daytime temperatures at which the plant normally grows (Raison et al, 1980). Respiration is a temperature dependent process having the Q_{10} of 2 to 2.5 between 5 and 25°C, however the rate of respiration is decreased at a much higher temperature (Salisbury and Ross, 1978). In P. fruticosa, the higher temperature produced faster growth of plant parts, with shoots and flower buds developing at a faster rate. The plants in the control, 15/5°C, showed very slow growth and ultimately some plants ceased growth and some leaves abscised after 27 weeks of the experiment.

The importance of photosynthate availability was shown by Biran and Halevy (1974a) using rose flowers. Conditions inducing low photosynthetic activity such as the removal or shading of leaves, decreased pigmentation of Baccara rose petals. A supplement of CO_2 to the atmosphere enhanced pigmentation as well (Biran et al, 1973).

Low temperature treatments enhanced pigmentation in many plants (Biran and Halevy, 1974a and 1974b; Biran et al, 1973; Creasy, 1968; Rutland, 1968). These treatments reduced both photosynthesis and respiration, however the reduction in respiration appears to be greater than that in photosynthesis (Q_{10} respiration > Q_{10} photosynthesis), resulting in greater carbohydrate availablilty at low temperature.

Anthocyanins are produced near the end of the flavonoid pathway. This may account for the restriction of their distribution only among the higher plants. Besides the indirect effect of high temperature through reduced available carbohydrate-controlling inhibition and activation of enzymes, there is a direct inhibition of anthocyanin synthesis by controlling enzyme synthesis.

The feedback control of enzyme activity is a very rapid mechanism, whereas the regulation of metabolic pathways by the repression and induction of enzymes is a relatively slow response (Hansen et al, 1967). The observed decline in anthocyanin synthesis is thought to involve both types of regulation. The immediate feedback control is difficult to determine on a whole flower since anthocyanins are synthesized throughout the bud development.

The carbohydrate availibility may also act as inducer and repressor of enzyme synthesis through the production of precursors.

The activity of enzymes is often measured to estimate the concentration of enzymes. Phenylalanine ammonia lyase (PAL) is a key enzyme in the phenyl propanoid pathway. Activity was measured in Euonymous alatus cv. Compactus leaves upon low temperature treatment (Creasy, 1974). There was an immediate increase in activity, followed by increases in flavonols, flavalans, and lastly anthocyanins. The maximum activity of PAL was reached about one week before maximum anthocyanin content.

PAL is an enzyme for many phenolic compounds and although a close correlation between PAL activity and anthocyanin synthesis is usually observed, it is not the only enzyme regulating anthocyanin synthesis. PAL was observed to have higher activity at low temperature, 6°C, accompanied by higher anthocyanin production in an apple skin, than at high temperature, 18°C, in the light (Tan, 1979). The lower PAL activity was accompanied by a higher PAL inactivating system. In the absence of light, (ie dark), PAL activity remained at a constant level, however there was little or no anthocyanin synthesis. In another study using apple skin discs, PAL activity in the dark was as high as in the light, but no anthocyanin accumulated (Faragher and Chalmers, 1977).

The lack of anthocyanin synthesis under high temperature may also be due to enzyme deactivation. Each enzyme has an optimum temperature for activity (Salisbury and Ross, 1978). Temperature increase resulted in a higher rate of protein denaturation. The higher temperature may destroy enzymes necessary for anthocyanin synthesis in *P. fruticosa*.

The temperature related changes of peroxidase isozyme activity and anthocyanin colouration of *Pedianthus tithymaloides* L. *variegatus* were reported by Bricage (1982). A fast moving group of isozymes was correlated with the development of anthocyanin pigmentation of the leaves. This group of isozymes occurred with a continuous large daily temperature difference. This is an example of temperature adaptation by isozymes. Peroxidases are an important group of enzymes responsible for oxidation activities.

The isozyme adaptability also varies among ecotypes of a species. The lowest Km values of malate dehydrogenase of two populations of *Potentilla glandulosa* occurred at temperatures each of which was near the average habitat temperature (Teeri and Peet, 1978). In contrast to *Pedilanthus*, the two populations of *P. glandulosa* showed very low adaptability to temperature, thus thought to be a genetic difference of two populations.

The variability of degree of temperature effect among the cultivars was observed during the field experiment. The possible presence of an isozyme system on anthocyanin synthesis similar to the malate dehydrogenase isozyme system is a desirable character in the selection program of P. fruticosa.

The point of control in the anthocyanin biosynthesis pathway was not studied. The measurements of intermediates such as soluble carbohydrates, cinnamic acid, and other flavonoids would assist in an understanding of the temperature effect.

Chlorophylls and carotenoids are found in plastids (Duysen and Freeman, 1974 and 1976; Goodwin, 1966; Simpson et al, 1975). Chromoplasts containing carotenoids are often developed from leucoplasts along with chloroplasts (Goodwin, 1966; Simpson et al, 1975) thus a close metabolic control over these two types of pigments is expected.

There were no detectable chlorophylls in the mature flowers of P. fruticosa taxa examined. However, petals in buds at stage 1, which appeared greenish, may have contained some chlorophylls. Sepals of Strelitzia reginae not containing a detectable amount of chlorophylls at maturity contained some chlorophylls at the earlier bud stage (Simpson et al, 1975).

The temperature effect on plastid pigment accumulation resulted in little effect on chlorophyll production whereas carotenoid production was maximal at 30°C and about one half the production at 15°C (Stickland, 1974a). A similar result was obtained in water stressed wheat leaves (Duysen and Freeman, 1974). The reduction of carotenoid content was greater than the chlorophylls three hours after the stress began.

The carotenoid concentration in _P_. _fruticosa_ flowers varied with the temperature. Higher temperature increased the concentrations in Orangeman, Tangerine, and UM7904, whereas an opposite effect was observed for Friedrichseni, UM7911, UM7513 and UM8105. The difference in response to temperature in _P_. _fruticosa_ may be due to the difference in the types of carotenoids present. _C_. _morifolium_ cv. Fandango florets (Stickland, 1974a) and UM7904 may have contained mostly carotenoids that increased with temperature and tomatoes examined by Goodwin (1966) and UM7911 may have contained the other types.

The effect of temperature on carotenoid concentration was smaller than that on anthocyanins, up to 86 percent reduction for carotenoids and up to 100 percent for anthocyanins. This suggests that the metabolic pathway of carotenoid synthesis is less sensitive to temperature. This may arise from carotenoids having a priority in obtaining carbohydrates over anthocyanins, and in turn chlorophyll synthesis is favoured over these. This may be a result of an evolutionary selection in that the presence of carotenoids is more important than anthocyanins. The temperature effect on anthocyanin and carotenoid synthesis showed a much smaller tolerance for anthocyanins, from 11 to 25°C, than for carotenoids, 6 to 35°C, which are in turn more affected than chlorophylls (Stickland, 1974a).

Carbohydrate or some other precursor availibility may be attributed to the change in carotenoid production. The much higher temperature for maximum carotenoid production, 38°C, in _C_. _morifolium_ cv. Fandango florets than for anthocyanins, 15°C, may be explained by the considerably lower sucrose concentration required for maximum carotenoid production, 0.6 to one percent, than for anthocyanins, one percent (Stickland, 1974a). The types of carotenoids involved are not reported in this study.

The _Potentilla_ species contained a high percentage of epoxy carotenoids and their derivatives (Schmid and Polaczek-Wittek, 1939; Valadon and Mummery, 1968a). They are synthesized at the advanced stages of the carotenoid synthesis pathway. It is possible that the temperature effect appeared to be less because the effect may have been at the oxidation process and the estimation of total carotenoid did not account for this difference. Further identification of pigments and quantitative analysis of each pigment will be required to clarify this point.

5.2 PETAL NUMBER

The field experiments indicated that high temperature around the time of flower initiation increased the petal number of some taxa. This finding was unexpected and is contrary to results on other flowers (Baer and Kho, 1971; Garrod and Harris, 1974; Reynold and Tampion, 1983). However, two different types of petal number increase were observed on carnation by both low and high temperatures (Garrod and Harris, 1974). These two types of increase appeared to be under different hormonal control. The petal number increase obtained by localized high temperature treatment was duplicated by an application of kinetin. Gibberellin synthesis is known to be promoted by low temperature in a wide range of plant materials (Reynold and Tampion, 1983). Petal number increase resulting from gibberellin application is similar to that under low temperature conditions (Garrod and Harris, 1974). Different types of gibberellins were shown to be responsible for determining double or single flowers in Nigella damascena (Raman and Greyson, 1977).

The field experiments also indicated that petal number decreased with increased plant water stress. These effects were more significant with taxa Sundance and UM7901, which had greater petal number change than the other taxa, Hersi and UM8102.

The temperature experiment under controlled environment resulted in no significant change of petal numbers among temperature treatments. This may indicate that the temperature effect occurred under field conditions only in conjunction with plant water stress.

The negative linear correlation between petal number and flower number obtained in the controlled environment study indicate that at the time of flower initiation, a competition exists between initiation of floral initial and petal primordia. A control mechanism governing this is unknown, however many studies indicated hormonal control (Abdul and Harris, 1978; Garrod and Harris, 1974; Moe, 1971).

The competition may have been on obtaining carbohydrates. Conditions inductive to higher photosynthetic activities promote flower initiation. High-light intensities increased the floret number on <u>Chrysanthemum</u> <u>morifolium</u> (Cockshull and Hughes, 1971). Earlier floral initiation was achieved on <u>Pelargonium</u> x <u>hortorum</u> receiving full light than those in 60 percent shade (Armitage and Watzstein, 1984).

The effect of temperature in combination with plant moisture status could have affected the photosynthetic activity of <u>P</u>. <u>fruticosa</u>. Higher temperature and greater water stress caused lower available carbohydrate, resulting in higher petal number and lower flower number.

Chapter VI

SUMMARY AND CONCLUSIONS

On the basis of the reseach conducted, the following can be concluded;

1. Flower colour
   - a) Pigment analysis
     - i) The pigments present in the petals were anthocyanins, carotenoids and flavonoids other than anthocyanins.
     - ii) The main pigment groups contained in different flower colours were;

       yellow : carotenoids
       salmon : anthocyanins, carotenoids and flavonoids
       pink : anthocyanins and flavonoids
       orange : anthocyanins and carotenoids
     - iii) The change of flower colour from cyanic to acyanic is due to reduction of anthocyanin synthesis.
   - b) Environmental effects
     - i) Anthocyanin concentrations in plants grown under field conditions were highest at the beginning and end of the flowering season and were very low during July and August.

ii) Linear regression analysis was carried out to determine the possible environmental cause of pigment concentration change. Temperature significantly affected anthocyanin concentrations of some cyanic taxa with greater effect by soil temperature than by air temperature. The concentration decreased with an increase in temperature.

iii) The degree of the effect varied among the 10 taxa examined.

iv) Carotenoid concentration in plants grown under field conditions varied over time. There was significant effect by temperature with greater effect by soil temperature than air temperature on some taxa.

v) The effect varied among the 10 taxa examined. Concentrations of some taxa increased with an increase in temperature whereas on some others an opposite effect was observed.

vi) There was no effect on anthocyanin and carotenoid concentrations by moisture factors.

c) Temperature effect

i) Under controlled environment conditions, anthocyanin concentration decreased with an increase in temperature. The decrease was greatest at 30/20°C treatment and the concentration was zero. The higher the treatment temperature, the greater and the faster the reduction of anthocyanin concentration.

ii) The effect of temperature on carotenoid concentration differed between the two selection lines examined. The higher temperature treatment resulted in higher concentration in one and lower in the other.

iii) The reduction of anthocyanin synthesis was most significant during the early stages of bud development, when most of the pigments were synthesized.

iv) The total carotenoids was greatly reduced at higher temperature (30/20°C) mainly due to the reduction in pigment synthesis during early bud development. The concentration reduction was less pronounced than with anthocyanin.

2. Petal number

a) Environmental effects

i) The petal number of four taxa under field conditions changed during the growing season.

ii) Petal number was affected by temperature about the time of bud initiation. Petal number increased with an increase in temperature.

iii) The degree of the temperature effect varied among the taxa examined.

iv) Among the moisture factors, there was no effect by precipitation. Petal number was significantly affected by plant water potential about the early bud development stage. Petal number decreased with an increase in water stress.

b) Temperature effects

i) Under controlled environment, there was no significant effect of temperature on petal number, thus the effect of temperature observed under field condition appears to be a combined effect with plant water potential.

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Appendix A

EVALUATION OF FLOWER PERIOD, COLOUR, AND PETAL NUMBER OF *POTENTILLA FRUTICOSA*

TAXA	FLOWER PERIOD (days)	COLOUR*	PETAL NUMBER
Abbotswood	107	1	5
Arbuscula	93	5	5
Beani	100	1	5
Beesi	85	3	5
Berlin Beauty	71	5	5
Bowflats Double	71	8	5
Bowflats One	107	7	5
Bowflats Two	85	8	5
Bowflats Three	85	7	5
Buttercup	120	8	5
Consolation Lake One	86	7	5
Consolation Lake Two	87	7	5
Consolation Lake Three	78	8	5
Consolation Lake Four	100	7	5
Consolation Lake Five	100	8	5
Coronation Triumph	100	7	5
Darts Golddigger	113	8	5
Darts Nugget	108	7	5
Daydawn	59	S	5
Elizabeth	85	5	5
Emma Lake	78	5	5
Fran Lady Daresbury	100	5	5
Farreri	113	7	5
Farreri White	107	1	5
Friedrichseni	85	5	5
Friesengold	100	5	5
Glabra	50	6	5
Gold Drop	100	8	5
Goldfinger	100	8	5
Goldfried	85	7	5
Grandiflora	113	8	5
Hackmans Giant	113	7	5
Hersi	85	1	EXP
Hurstbourne	115	7	5
Irving	127	7	5
Jackmanni	86	8	5
Katherine Dyke	87	4	5
Knaphill	127	8	5
Lemondrop	114	5	5

TAXA	FLOWER PERIOD (days)	COLOUR*	PETAL NUMBER
Longacre	100	5	5
Maaneleys	100	4	5
Mandshurica	43	1	5
Marchand	115	8	5
Micandra	114	8	5
Moonlight	107	5	5
Mount Everest	94	1	5
No. 700	85	5	5
Northman	115	6	5
Nyewood Form	65	7	5
Ochroleuca	78	3	5
Orangeman	115	0	5
Parvifolia	113	2	5
Peyto Lake One	93	8	5
Peyto Lake Two	107	7	5
Peyto Lake Three	86	7	5
Peyto Lake Four	87	7	5
Peyto Lake Five	100	7	5
Peyto Lake Six	107	7	5
Peyto Stream	63	7	5
Peyto View	114	7	5
Primrose Beauty	108	3	5
Purdomi	107	4	5
Rhederiana	93	4	5
Roseacre	108	1	5
Sandved	63	6	5
Subalbicans	85	1	5
Sulphurescens Logan Form	120	8	5
Sundance	108	4	EXP
Sunset	56	0	5
Sutters Gold	85	5	5
Tangerine	85	0	5
Tenuiloba	64	8	5
Veitchi	107	1	5
White Gold	85	5	5
Whitemouth	71	8	5
Yellow Single	107	4	5
UM7101	115	0	5
UM7102	108	4	EXP
UM7103	71	2	5
UM7301	120	7	EXP
UM7302	100	0	5
UM7303	85	0	5
UM7304	78	8	5
UM7305	63	0	5
UM7306	92	7	5
UM7307	63	7	5
UM7309	113	1	5

TAXA	FLOWER PERIOD (days)	COLOUR*	PETAL NUMBER
UM7501	56	6	5
UM7502	63	8	EXP
UM7503	105	2	EXP
UM7504	105	3	5
UM7505	56	S	5
UM7506	105	O	5
UM7507	56	1	5
UM7508	56	O	5
UM7509	105	4	EXP
UM7510	85	5	5
UM7513	63	S	5
UM7514	85	O	5
UM7515	85	O	5
UM7516	85	O	5
UM7517	93	1	5
UM7518	108	1	5
UM7519	92	7	5
UM7520	100	5	5
UM7521	92	2	5
UM7522	100	P	5
UM7523	100	P	5
UM7524	56	O	5
UM7525	63	O	5
UM7528	105	6	5
UM7901	85	7	EXP
UM7902	92	2	EXP
UM7903	107	1	EXP
UM7904	114	O	5
UM7905	126	7	5
UM7906	85	7	5
UM7907	100	8	EXP
UM7908	120	7	5
UM7909	120	4	EXP
UM7910	120	6	5
UM7911	120	P	5
UM7912	85	5	5
UM7913	63	2	5
UM7914	75	6	5
UM7915	56	O	5
UM7916	120	1	5
UM7917	100	2	5
UM7918	63	O	5
UM7920	92	O	EXP
UM7921	92	S	EXP
UM7922	120	O	5
UM7923	100	8	5
UM7924	100	S	5
UM7925	107	4	EXP

TAXA	FLOWER PERIOD (days)	COLOUR*	PETAL NUMBER
UM7926	71	P	5
UM7927	120	7	EXP
UM7928	78	S	5
UM7929	85	S	5
UM7930	100	P	5
UM7931	71	O	5
UM7932	56	5	EXP
UM7933	80	O	5
UM7950	105	3	EXP
UM7951	63	3	5
UM7952	-	5	5
UM7953	79	2	5
UM7954	120	6	EXP
UM7955	93	5	EXP
UM7956	93	O	5
UM7957	64	3	5
UM7959	87	5	EXP
UM7960	78	5	EXP
UM7961	100	5	5
UM7962	93	7	5
UM7963	115	7	5
UM7964	64	4	5
UM7965	64	7	EXP
UM8101	115	5	5
UM8102	122	1	EXP
UM8103	120	6	EXP
UM8104	63	6	5
UM8105	50	O	5

*dominant flower colour during the growing season according to the classification of flower colours

Appendix B

DAILY MAXIMUM AND MINIMUM AIR AND SOIL TEMPERATURES, THE UNIVERSITY OF MANITOBA, 1983

June

	air temperature (°C)		soil temperature (°C)	
date	maximum	minimum	maximum	minimum
1	22.0	6.0	13.0	8.0
2	22.0	12.0	12.0	9.5
3	20.0	12.0	14.5	10.0
4	14.5	10.0	12.0	9.0
5	19.0	5.0	12.0	7.0
6	23.5	7.0	14.0	8.0
7	27.0	6.0	15.0	8.0
8	19.0	11.0	14.0	11.0
9	27.0	6.0	15.5	9.5
10	33.0	17.0	18.0	13.0
11	32.0	21.0	19.0	15.0
12	25.5	13.0	18.0	15.5
13	21.0	14.5	16.5	15.0
14	23.0	13.0	17.0	13.0
15	12.0	8.0	15.5	12.0
16	20.0	6.0	15.0	10.5
17	24.5	10.0	17.0	12.0
18	25.5	10.0	17.0	13.0
19	24.5	16.5	16.5	14.5
20	26.5	19.5	19.0	15.5
21	28.0	16.5	19.5	15.5
22	24.5	15.5	20.5	16.5
23	26.5	21.1	23.0	14.5
24	29.0	14.5	22.0	15.5
25	26.5	21.0	23.0	18.0
26	24.0	14.5	23.0	16.5
27	23.0	10.0	22.0	14.5
28	26.0	11.0	23.0	15.0
29	24.5	12.0	20.0	15.5
30	19.0	14.5	18.0	16.0

July

	air temperature (°C)		soil temperature (°C)	
date	maximum	minimum	maximum	minimum
1	22.0	15.5	20.0	15.5
2	18.0	15.5	18.0	16.0
3	25.5	18.0	22.0	16.5
4	18.0	11.0	18.0	15.5
5	20.0	6.5	20.0	12.0
6	29.0	12.0	23.0	14.5
7	30.0	18.0	25.0	18.0
8	32.0	20.5	26.0	19.5
9	30.0	20.0	24.5	20.0
10	33.0	22.0	26.5	20.5
11	24.5	16.0	22.0	19.0
12	32.0	14.5	25.0	18.0
13	31.5	20.5	25.0	19.5
14	34.0	18.0	26.5	19.0
15	33.0	22.0	26.5	21.0
16	26.0	18.0	25.0	21.0
17	28.0	14.5	24.5	18.0
18	29.0	15.5	24.5	19.0
19	29.0	18.0	25.0	19.5
20	30.5	17.0	26.0	19.5
21	32.0	19.0	26.5	20.0
22	32.0	18.0	25.5	20.0
23	24.5	18.0	23.0	19.5
24	28.0	16.5	24.5	18.0
25	29.5	14.5	25.5	18.0
26	31.0	20.5	24.0	20.0
27	31.0	22.0	26.5	20.5
28	32.0	20.5	26.0	20.5
29	30.0	19.0	26.0	20.5
30	29.0	14.5	25.5	20.0
31	28.0	17.0	23.0	18.0

August

date	air temperature (°C) maximum	air temperature (°C) minimum	soil temperature (°C) maximum	soil temperature (°C) minimum
1	31.0	17.0	25.5	18.0
2	32.0	20.0	25.5	20.0
3	34.0	19.5	28.0	20.0
4	37.0	22.0	28.0	21.0
5	33.5	22.0	26.0	22.0
6	34.5	14.0	25.0	19.0
7	33.5	23.0	25.5	20.5
8	28.0	16.5	24.5	19.0
9	30.0	19.0	24.0	19.5
10	26.5	16.0	24.5	19.0
11	30.5	15.0	24.5	18.0
12	33.5	15.0	24.5	19.5
13	30.0	19.0	24.0	19.0
14	33.5	13.5	23.5	18.0
15	33.5	22.0	24.5	19.5
16	23.0	17.0	21.0	19.0
17	36.5	14.5	24.0	17.0
18	37.0	18.0	24.0	19.0
19	26.5	18.5	22.0	18.5
20	18.5	14.5	18.0	16.5
21	26.0	15.0	22.0	16.5
22	26.5	13.0	22.0	15.5
23	28.0	17.0	21.0	17.0
24	30.0	18.0	23.0	17.0
25	34.5	19.5	24.0	18.5
26	32.0	19.5	24.0	19.0
27	33.5	17.0	24.0	18.5
28	32.0	17.0	23.5	18.5
29	31.0	18.5	24.0	19.0
30	31.5	16.0	23.5	18.5
31	35.0	19.5	23.5	19.0

September

	air temperature (°C)		soil temperature (°C)	
date	maximum	minimum	maximum	minimum
1	35.0	21.0	23.5	19.5
2	39.5	19.0	24.0	19.0
3	29.5	19.0	21.0	18.5
4	29.5	14.5	21.0	16.5
5	23.0	13.0	19.0	16.5
6	18.0	12.0	16.5	15.0
7	26.0	9.0	19.5	13.5
8	20.0	14.5	16.0	15.0
9	23.0	12.0	18.0	13.0
10	17.0	13.5	15.5	13.5
11	18.5	8.5	15.5	9.5
12	19.0	8.0	16.5	10.0
13	18.0	9.0	15.5	11.0
14	19.5	4.0	15.5	9.0
15	15.0	13.0	13.5	11.0
16	16.0	12.0	14.5	11.0
17	19.0	13.0	14.0	11.0
18	15.5	11.0	12.0	10.0
19	12.0	5.5	11.0	7.0
20	10.0	8.0	10.5	8.5
21	10.0	5.0	10.5	7.0
22	9.5	1.5	10.0	6.0
23	20.0	1.5	11.0	5.5
24	24.5	9.0	13.0	7.0
25	24.5	9.5	14.0	9.5
26	27.0	8.0	15.0	9.0
27	31.0	12.0	15.5	10.5
28	26.5	9.0	14.5	10.5
29	9.5	4.0	11.0	8.5
30	8.5	5.0	9.5	8.5

October

date	air temperature (°C) maximum	air temperature (°C) minimum	soil temperature (°C) maximum	soil temperature (°C) minimum
1	11.0	7.0	10.0	8.5
2	9.5	6.0	9.5	8.0
3	12.0	9.0	10.0	9.0
4	13.5	7.0	10.5	9.0
5	12.0	4.0	9.5	6.5
6	13.0	2.0	9.0	5.0
7	11.0	3.5	10.5	4.5
8	16.0	2.0	9.0	5.0
9	15.5	5.5	9.0	4.5
10	17.0	10.0	10.5	8.0
11	6.5	3.5	7.0	6.5
12	5.5	1.5	6.0	4.5
13	8.0	-2.0	6.0	3.0
14	12.0	-1.0	6.5	3.0
15	9.0	0.5	5.5	3.5
16	6.5	3.0	5.5	14.0
17	7.0	-3.5	4.5	2.0
18	6.0	-4.0	4.5	1.0

Appendix C

MOISTURE RECORDING, THE UNIVERSITY OF MANITOBA, 1983

date		weekly precipitation (mm)	plant water potential (atoms)
June	21	19.9	-12.37
	28	23.0	-4.47
July	5	34.6	-11.96
	12	0	-16.37
	19	37.5	-14.51
	26	0	-12.98
August	2	4.3	-17.58
	9	8.4*	-14.45
	16	0	-15.70
	23	19.7	-16.01
	30	15.9	-17.90
September	6	1.8	-12.14
	13	15.1	-16.71
	20	6.2	-13.78
	27	11.2	-17.55
October	4	26.9	-19.79

*includes irrigation