CHAPTER 6

EFFECTS OF VARIOUS INDUCTIVE PERIODS AND CHEMICALS ON FLOWERING AND VEGETATIVE GROWTH OF TOMMY ATKINS AND KEITT MANGO CULTIVARS.

6.1 ABSTRACT

The effects of potassium nitrate and paclobutrazol on flowering and vegetative growth of 'Tommy Atkins' and 'Keitt' mango were studied under various periods of exposure to inductive and non-inductive temperature regimes. The experiment was done at the University of Pretoria experimental farm in temperature-regulated cabinets on 2-year-old potted 'Tommy Atkins' and 'Keitt' mango cultivars. 'Keitt' was more sensitive towards low temperature floral induction than 'Tommy Atkins'. For both cultivars, the trend revealed that, incomplete floral induction could be complemented by paclobutrazol application. Paclobutrazol also significantly reduced vegetative growth and number of days required for a visible inflorescence emergence in both cultivars. Potassium nitrate promoted the sprouting of buds for vegetative growth under non-inductive temperature conditions and reproductive growth under inductive conditions. The minimum inductive period at 10/15^{o}C (12 h light/12 h dark) required for "complete" floral induction and development was found to be 35 days for both cultivars.

Surpassing the inductive (cold) period showed adverse effects on normal development of the reproductive parts and also delayed inflorescence emergence.

Key words: cold units, flowering, paclobutrazol, potassium nitrate, vegetative flush

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6.2 INTRODUCTION

Induction refers to the commitment of buds to evoke a particular shoot type, i.e., vegetative shoot (vegetative induction), generative shoot (floral induction) or mixed shoot (combined vegetative–floral induction) (Davenport & Nunez-Elisea, 1997). Floral induction of most plants involves sensing some environmental cue, i.e., day length, water stress, or vernalising temperature in some organs (Davenport & Nunez-Elisea, 1997). The event is translated to the production of a putative floral stimulus or alteration in the ratio of florigenic and anti-florigenic components that may be translated to target cells in meristems (Bernier *et al*., 1981). However a specific compound that acts as a floral stimulus has never been isolated, casting doubt on its existence (Lang *et al*., 1977).

Studies in mango, on the other hand, revealed the existence of a floral stimulus, which is continuously synthesised in mango leaves during exposure to cool, inductive temperatures (Davenport & Nunez-Elisea, 1990). Unlike other plants requiring vernalisation for induction (Bernier *et al*., 1981), mango leaves appear to be the only site where the putative floral stimulus is produced (Nunez-Elisea & Davenport, 1992). Complete defoliation of girdled branches during inductive conditions results in vegetative shoots instead of generative shoots (Nunez-Elisea & Davenport, 1991b; Nunez-Elisea & Davenport, 1992). The putative temperature regulated floral stimulus is short-lived *in situ*, its influence only last 6-10 days (Nunez-Elisea & Davenport, 1992; Nunez-Elisea *et al*., 1996). Temperatures below 15^{o}C readily promote floral induction, whereas vegetative growth is generally promoted by warmer temperatures (Whiley *et al*., 1989; Nunez-Elisea & Davenport, 1991b). Ravishankar *et al*. (1979)

however, found that low temperature appears to exert a depressing effect on the further development of flower buds of mango. Under field conditions, the duration of cool inductive temperature (cold units) for reproductive morphogenesis might not be sufficient as required by a specific cultivar, or may revert from inductive to non-inductive conditions before complete floral induction is achieved. These conditions only favour partial floral induction or complete vegetative morphogenesis. This is why attainment of floral induction does not ensure initiation of floral morphogenesis (Nunez-Elisea & Davenport, 1995). According to the latter authors, growth of induced buds in the presence of cool temperature was found to be essential for floral initiation (resumed growth), because insufficiently induced apical buds that resumed growth after trees were transferred to warm temperatures outdoors, produced a vegetative flush instead of an inflorescence.

Initiation of apical buds was stimulated at the start of temperature treatment by defoliating shoot tips (Nunez-Elisea & Davenport, 1991b). Bud initiation (resumed growth) was characterised as the swelling and initial elongation of the apex (about 5mm in height), which assures a distinct conical shape, and had tightly clasped outer bud scales (Nunez-Elisea *et al*., 1993). Bud break is considered the stage at which external bud scales loosened and began to open (Nunez-Elisea & Davenport, 1991a).

Growth regulators should be assessed for their complemental or total substitution effects (for some cultivars) of cold temperature requirement for reproductive morphogenesis. Positive results with growth regulators may have special attributes in places with poor floral inductive climatic conditions or with frequent and sudden changes in temperature for sufficient floral induction. In countries like Ethiopia, such

incomplete floral induction is often experienced and sometimes leads to crop failures. Hence, this experiment was designed to determine the ability of paclobutrazol (PBZ) and potassium nitrate (KNO_3) to complement or intensify flowering and also their effect on vegetative growth. A field experiment using the same chemicals was simultaneously done in Ethiopia.

6.3 MATERIALS AND METHODS

6.3.1 Area description and season

The experiment was done at the University of Pretoria experimental farm in temperature-regulated chambers. The growth chambers provided a photosynthetic photon flux of 334-399 μmol m^{-2} s^{-1} at the upper tree canopy level and a relative humidity above 75%. The experiment began in early December 2002 when the average day/night temperatures were around 31/16^{o}C and therefore non-floral inductive.

6.3.2 Plant materials

Uniform 2-year-old potted Tommy Atkins ('TA') and Keitt ('KT') mango trees were used for the experiment. The plants were decapitated by removing the terminal 2-3 cm of the stem, including the terminal bud, to induce a new vegetative flush before the onset of the experiment. The plants were watered every third day and received 250 ml of a standard Hoagland solution once a week. The trees had almost equal numbers of leaves ($\pm$10) and not more than 2 flushes per tree.

6.3.3 Treatments and experimental design

The experiment was a three factor factorial combination in a randomised complete block design with three replications. Randomly assigned three plant replications per treatment were used to apply four chemical treatments (no treatment (control), 3% potassium nitrate (KNO_3), 500 ppm paclobutrazol (PBZ) or 2000 ppm PBZ) in a factorial combination. The trees were kept under inductive temperature conditions (15/10^{o}C 12 h/12 h light/dark) for four durations, 0 (control), 15, 35, or 60 days. After the trees were induced with cold treatment for the above-mentioned durations, they were transferred to a non-inductive warm temperature cabinet (25/20^{o}C).

Due to limited space per chamber, three cabinets of the same make, temperature and light settings were used.

6.3.4 Parameters recorded

The number of days the trees were exposed to a low temperature regime for each treatment was taken as the number of cold units (Robbertse & Manyaga, 1998). The date of flowering (beginning of flowering) was recorded as the number of days passed after first spray and/or beginning of inductive or non-inductive temperature treatment to the production of a visible inflorescence (bud break stage). Only trees that were subjected to the low temperature for 35 and 60 days (both sprayed with the chemical treatments or non-sprayed) were compared since all trees in the three replications flowered. It was difficult to compare the other treatment combinations since all or most

of their replicated trees did not flower. To detect differences in flowering time in relation to treatments, inflorescence development was monitored every day.

The number and length of the inflorescences produced was recorded at the end of the experiment. The number and surface area (length (cm) x width (cm) = cm^2), of any new leaves that were produced and length of new flushes (cm) produced were also recorded. The total duration of the experiment was 130 days.

6.3.5 Statistical analysis

Logarithmic transformations were done where necessary, to normalise a highly variable data set before data analysis. Statistical analysis was performed using the Genstat, (2000) computer package (release 2.2) and comparison of means was done using Least Significant Difference (LSD) at 5% level of significance. Flowering response was analysed using a General Linear Model (GLM) for unbalanced designs (Joubert *et al*., 1993).

6.4 RESULTS

6.4.1 Numbers of inflorescences produced

Regardless of the cultivars, the numbers of inflorescences developed were affected both by the time the trees were kept under inductive condition and by chemical spray (Tables 6.1 and 6.2). Duration of the inductive temperature for 35 days and above had significantly increased the number of inflorescences developed per tree as compared to

the control for both cultivars (Table 6.1). Trees that were under inductive temperature for 60 days had significantly higher number of inflorescences than all the other exposure periods (Table 6.1, Fig. 6.1). On the other hand, even if the interaction between duration and chemicals was not significant, the trend showed that both 'TA' and 'KT' trees sprayed with 500 and 2000 ppm PBZ did flower, where the supplemental chemical spray was applied to trees exposed to the inductive temperature for only 15 days (Table 6.2). Non-sprayed and trees that were sprayed with 3% KNO_3 did not flower after 15 days stay in the inductive temperature. Averaged across cultivars and duration period in the inductive temperature, on the other hand, trees sprayed with 2000 ppm PBZ had a significantly higher number of inflorescences than the control as well as from trees sprayed with 3% KNO_3. The result, however, was not significantly different to spraying with 500 ppm PBZ. (Table 6.3, Fig. 6.2, 6.3 A&B). There was a 55.42% increase in the number of inflorescences developed when trees were sprayed with 2000 ppm compared with the control.

Table 6.1 Effect of different exposure periods to the inductive temperature on flowering and vegetative growth parameters

Duration in the inductive temperature (days)	Number of inflorescences	Length of new flushes (cm)	Number of new leaves developed	Leaf size (cm^2)
0 (control)	0.08c	20.07a	10.50a	86.91a
15	0.08c	17.12b	8.00b	77.63a
35	1.87b	11.67c	6.29bc	63.80b
60	2.21a	8.62d	4.87c	52.63b

Means followed by different letters in a column are significantly different by LSD test at P<0.05

Table 6.2 **Effects of various duration in the inductive temperature and chemicals on flower development of Tommy Atkins and Keitt mango cultivars.**

	Chemicals			
Duration (days)	0 (no chemical)	3% KNO_3	500 ppm PBZ	2000 ppm PBZ
0	0.00a	0.17a	0.00a	0.17a
15	0.00a	0.00a	0.17a	0.17a
35	1.33a	1.50a	2.17a	2.50a
60	2.00a	2.17a	2.33a	2.33a

Means followed by different letters in columns and rows are significantly different by LSD test at P<0.05

Figure 6.1 **'Tommy Atkins' trees exposed for 60 days to inductive temperature resulted in inflorescence development without additional chemical spray.**

Table 6.3 The effect of different chemical treatments on flowering and vegetative growth of 'Tommy Atkins' and 'Keitt'

Chemical treatments	Number of inflorescences per tree	Days from first day of induction to bud break	Length of new flushes (cm)	Number of new leaves developed per tree	Leaf size (cm^2)
0 (control)	0.83c	114.8a	17.05b	7.00b	74.78b
3% KNO_3	0.96bc	103.5b	21.54a	9.62a	89.76a
500ppm PBZ	1.17ab	96.25c	11.64c	7.35b	66.66b
2000ppm PBZ	1.29a	90.58d	7.24d	5.67b	49.76c

Means followed by different letters in a column are significantly different by LSD test at P<0.05

Figure 6.2 Inflorescence development in 'Tommy Atkins' trees after 35 days induction and application of 500 ppm paclobutrazol.

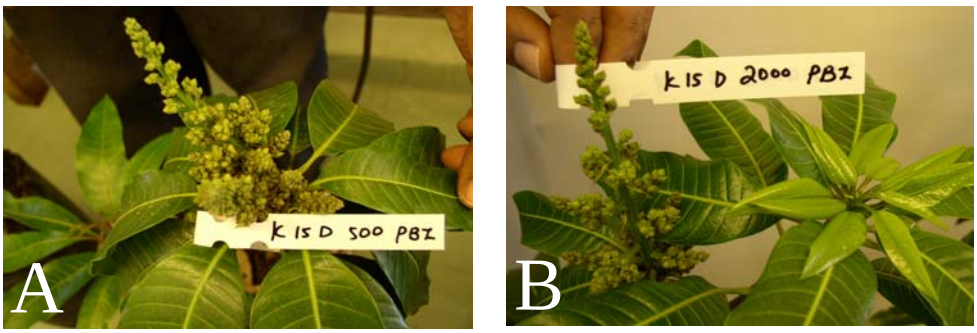

Figure 6.3 Paclobutrazol at 500 ppm (A) and 2000 ppm (B) concentration complemented the flower induction process in 'Keitt' trees that were exposed for only 15 days to inductive temperature.

6.4.2 Length of inflorescences produced

There was a significant difference for the interaction among cultivars, duration in the inductive temperature, and chemical spray for the length of inflorescences developed (Table 6.4). 'TA' trees that stayed 35 or 60 days under inductive temperature and were sprayed with 3% KNO_3 produced significantly longer inflorescences (25.5 cm) compared with the control (Table 6.4, Fig. 6.4). In 'KT' trees, the longest inflorescences were observed for trees that stayed 35 days under inductive temperature and were sprayed with 3% KNO_3 , even if the result was not significantly different to that of the control. Most of the trees that were not induced had no inflorescences at all and those sprayed with PBZ produced only short inflorescences (Fig. 6.5).

Figure 6.4 **Potassium nitrate at 3% concentration resulted in a significant increase in the length of inflorescence in 'Keitt' trees after the trees were exposed for only 35 days to inductive temperature (as for 'Tommy Atkins').**

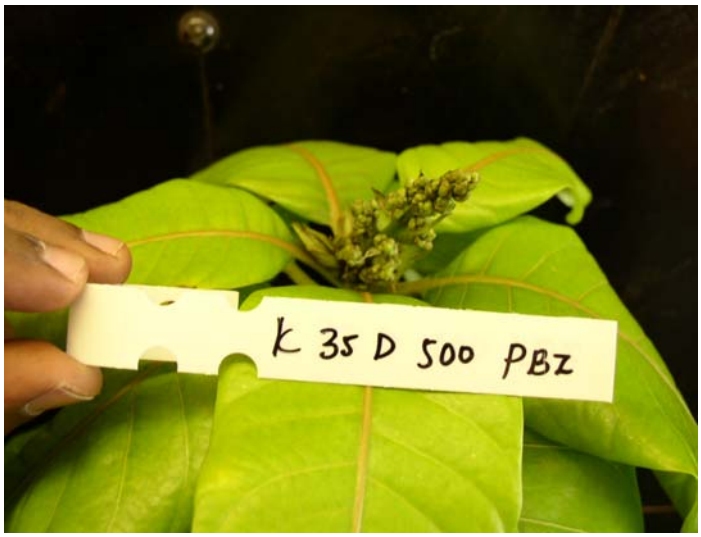

Figure 6.5 **Short inflorescences developed by spraying paclobutrazol unlike Potassium nitrate sprays in 'Keitt' trees.**

Table 6.4 **Effect of cultivars, varying induction periods and chemicals on the length of inflorescences (cm) in two mango cultivars**

Cultivar	Duration (days)	Chemical			
		0	3% KNO_3	500 ppm PBZ	2000 ppm PBZ
'TA'	0 (control)	0.00e	0.00e	0.00e	4.17e
	15	0.00e	0.00e	0.00e	0.00e
	35	15.87cd	25.50a	19.37a-d	16.27bcd
	60	20.00a-d	22.37ab	18.47a-d	15.43bcd
'KT'	0 (control)	0.00e	22.47ab	5.13e	0.00e
	15	0.00e	0.00e	5.07e	5.83e
	35	21.97abc	22.67ab	17.17bcd	13.60d
	60	21.97abc	21.27abc	17.67bcd	14.67cd

Means followed by different letters in columns and rows are significantly different by LSD test at P<0.05

6.4.3 Days from first day of induction to floral bud break

There was a significant interaction between cultivars and duration of low temperature treatment with respect to number of days required for floral bud break (Table 6.5). Chemical spraying also significantly affected the days required for floral bud break (Table 6.3). Both 'KT' and 'TA' trees that were exposed to the inductive temperature for 60 days required less number of days for flower bud break compared with trees that stayed only 35 days. The results also showed that 'KT' trees were induced and reacted to floral bud break prior to 'TA' trees. Bud break on trees sprayed with PBZ at a concentration of 2000 ppm was significantly advanced compared with control trees as well as trees of other treatments. It was observed from the current experiment that within 35 and 60 days inductive temperature period, the number of days elapsed for inflorescence emergence, declined from non-sprayed to KNO_3 and from KNO_3 to PBZ sprayed in both cultivars (Table 6.3).

Table 6.5 Reaction of 'TA' and 'KT' to two low temperature exposure periods on days required for floral bud break from the day of treatment application

	Duration (days)	
Cultivars	35	60
'Tommy Atkins'	112.75a	107.75b
'Keitt'	101.00c	83.58d

Means followed by different letters in columns and rows are significantly different by LSD test at $P<0.05$

6.4.4 Vegetative growth

The lengths of new vegetative flushes were affected by both the duration of exposing the trees to the inductive temperature and by the chemical treatments (Tables 6.1, 6.2). Irrespective of the cultivars used, the longer the trees were exposed to the inductive temperature, the shorter were the new vegetative flushes (Table 6.1). Consequently, the trees induced for various periods had significantly shorter vegetative flushes than the control (non-induced) (Fig. 6.6 A&B). Generally, there was a decrease in the length of new flushes developed from KNO_3 sprayed to the control trees and then from lower to higher PBZ concentration spray. In both cultivars, significantly longest flushes were observed on trees that were sprayed with 3% KNO_3 (Table 6.3, Fig. 6.7) (21.54 cm) and the shortest on trees sprayed with 2000 ppm PBZ (Table 6.3, Fig. 6.3B) (7.24 cm).

Figure 6.6 **'Tommy Atkins' (A) and 'Keitt' (B) trees that remained under non-inductive condition for the course of the experiment had longer vegetative flushes and more leaves.**

Figure 6.7 Longest new vegetative flushes were observed on trees sprayed with 3% potassium nitrate in 'Keitt' trees.

The number of new leaves per flush was affected by the duration in the inductive temperature and by chemical spray, independently (Tables 6.1, 6.2). In both cultivars, the induced trees had significantly shorter flushes with fewer new leaves compared with the control trees (Table 6.1, Fig. 6.6 A&B). Trees induced for 60 days had 115.61% reduction in new leaf development over the control. Averaged across cultivars and the duration period in the inductive temperature, trees sprayed with 3% KNO_3 produced a significantly higher number of leaves as compared with all the other spraying treatments (Table 6.3).

The size of the newly developed leaves was also affected by the nature of the cultivar, duration in inductive temperature, and chemicals sprayed, independently. Averaged across duration and chemical treatments, 'KT' trees had a significantly greater leaf size (76.47 cm^2) than 'TA' trees (64.02 cm^2) (Table 6.6). Keeping trees under inductive temperature for 35 or 60 days significantly reduced leaf size by approximately 41.31%

compared with the control, as well as trees that were induced for 15 days (Table 6.1, Fig. 6.8). Averaged across cultivars and duration in the inductive temperature, where trees were sprayed with 3% KNO_3 had a significantly greater size of newly developed leaves (by 20 % higher) than the control trees (Table 6.3). On the contrary, application of PBZ especially at a concentration of 2000 ppm significantly reduced the size of newly developed leaves (Table 6.3, Fig. 6.9).

Table 6.6 Effect of cultivar differences on the size of the newly developed leaves

Cultivars	Leaf size (cm^2)
'Tommy Atkins'	64.015a
'Keitt'	76.467b

Means followed by different letters in a column are significantly different by LSD test at P<0.05

Figure 6.8 Larger size of newly developed leaves is obtained for trees with no or lower number of exposure days in the inductive temperature in 'Tommy Atkins'.

Figure 6.9 Paclobutrazol at 2000 ppm concentration highly reduced the size of newly developed leaves in 'Keitt'.

6.5 DISCUSSION

Critical low temperature requirement and the minimum duration thereof, required for flower induction is determined by visible floral differentiation, which may be variable in different cultivars (Chaikiattiyos *et al.*, 1994). In the current experiment, the minimum number of cold units required for effective floral induction was found to be 35 days for both cultivars. Although the interaction between the duration and chemicals was not significant, the trend showed that PBZ applied at either 500 or 2000 ppm concentration had the potential to complement the cold temperature requirement for floral induction.

During the first round of this experiment that was done during the previous season (not described in this report) showed very similar results but due to unacceptable variations caused by defects in the growth cabinets, the data are not presented. Therefore, there

seems to be a practical possibility of using PBZ for better floral induction under poor inductive conditions as often experienced in Ethiopia. This statement can be supported by the results of a field experiment done (Chapter 4 in this thesis). In addition flowering intensity was increased both by longer duration of the trees under inductive temperature and by spraying trees, particularly by increasing the concentration of PBZ up to 2000 ppm. PBZ, owing to its anti-gibberellin activity (Quinlan & Richardson, 1984), could induce or intensify flowering by blocking the conversion of kaurene to kaurenoic acid. From the current experiment, a combination of an inductive temperature for 35 days and spraying of 500 ppm PBZ on the trees is found to be sufficient and economical for successful floral induction and higher flowering intensity.

Applications of higher concentrations of PBZ had a negative effect with regard to length of both inflorescence and vegetative growth of the trees, as well as number and size of new leaves developed. According to Steffens *et al.* (1985), PBZ has the greatest effect on immature tissues, which are still growing and differentiating. This could explain why PBZ affected predominantly the apical growth. Gao *et al*. (1987) also indicated that triazoles reduce leaf area. The result of a decrease in the vegetative growth parameters resulting from PBZ application in the current study agrees with that of Salazar-Gracia & Vazquez-Valdivia (1997); Hoda *et al*. (2001).

The increase in length of inflorescences and new flushes in response to KNO_3 treatment may be a result of the enhanced cell division and enlargement in the meristematic zones. A similar growth pattern was observed for the vegetative growth parameters of trees that stayed in the non-inductive temperature for long periods. Surpassing the required inductive cold period also hindered and delayed normal

inflorescence development. Hence, as indicated above, induction periods of 35 days or less may be sufficient for successful floral induction without adversely affecting the development of the reproductive parts. Ravishankar *et al.* (1979) also found that low temperature appears to exert a depressing effect on further development of flower buds of mango. A negative correlation occurred between number of inflorescence and length of flushes developed (r=-0.96*). The regression graph for the two parameters indicates the antagonistic development pattern of the two important plant parts (Fig. 6.10). According to Wolstenholme & Hofmeyer (1985), vegetative growth and fruiting in mango trees are largely antagonistic and that excessive vegetative growth, especially if there is no marked dry season, is likely to cause poor yields.

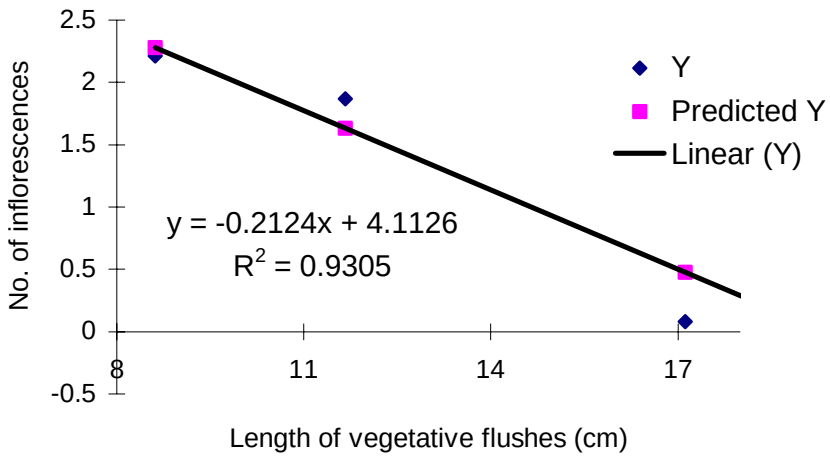

Figure 6.10 Regression between number of inflorescences developed and length of new vegetative flushes produced.

There was a significant positive correlation (r=0.98) between number of leaves and length of flushes developed. A regression graph of the two parameters signifies the direct relationship between flush length and number of nodes for leaf development (Fig. 6.11). From a pruning perspective, the direct relationship between flush length and leaf number implies a direct relationship between flush length and number of axillary bud per flush, thus increasing the scope for subsequent pruning.

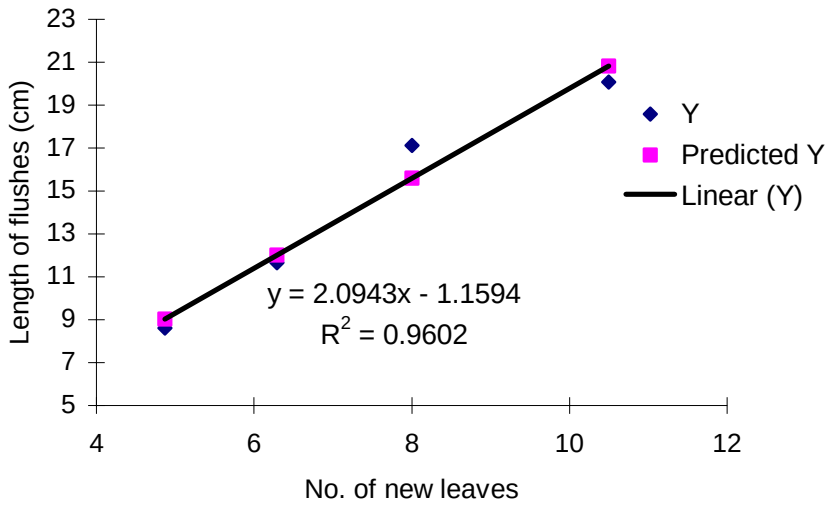

Figure 6.11 Regression between length of new vegetative flushes and number of new leaves developed.

Trees sprayed with 2000 ppm PBZ had their floral bud break 91 days after treatment applications (96 days for 500 ppm) whereas the control required 115 days. Thus, trees sprayed with the 2 PBZ concentrations advanced flowering by 22 days on average. It is probable that the application of PBZ caused an early reduction of endogenous gibberellin levels within the shoots as also observed by Anon (1984), causing them to

reach maturity earlier than those of untreated trees. The interaction between cultivar and duration revealed that 'KT' trees that stayed 60 days in the inductive temperature had their floral bud break 24 days earlier than 'TA'. This was also observed under field conditions on panicle pruning experiments conducted in South Africa (Chapter 5 in this thesis). In the latter study, it was revealed that even if flower induction and inflorescence development was earlier in 'KT' than 'TA' trees originally, the stage of fruit development in 'KT' became very slow to end up with an early fruit development and maturation in 'TA'. That situation proved the normal physiological characteristics of 'KT' to be a late cultivar. The effect of 500 ppm PBZ on the reduction of days for floral bud break may be sufficient as against applying 2000 ppm PBZ. This depends on the market situation for an early crop and economic analysis for the benefit of the two concentrations in a given country and orchard. The effect of PBZ on the reduction of days for inflorescence bud break in this experiment is similar to the observation of Tongumpai *et al*. (1996). On the other hand, the results for the floral and vegetative parameters considered in this trial are similar to the experiments of Nunez-Elisea & Davenport (1991a; b); Davenport & Nunez-Elisea (1997).

6.6 CONCLUSION

In general, bud development in to either a vegetative or a reproductive plant part is determined by the temperature to which the plants are exposed as reported by Nunez-Elisea (1985). In the current experiment, the minimum number of cold units required for sufficient floral induction was found to be 35 days for both cultivars. Nevertheless, PBZ application at 500 or 2000 ppm concentration also showed the potential to complement cold temperature requirement for trees that stayed only 15 days at floral

inductive temperature. It was also observed that the impact of KNO_3 and PBZ on various parameters is considerable. Applications of 3% KNO_3 spray in combination with a minimum inductive period of 35 days had a significant effect on increasing the length of inflorescences, especially in 'TA'. It can be deduced from the current experiment that spraying of 500 ppm PBZ may be sufficient to suppress vegetative growth that will in turn have an impact in encouraging reproductive growth. It was also found to be successfully sufficient to reduce the number of days for attaining floral bud break.