

Full Length Research Paper

The influence of exogenously applied 2,4-dichlorophenoxyacetic acid on fruit drop and quality of navel oranges (*Citrus sinensis* L.)

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Orchard experiments were carried out in Botswana with the objective of evaluating the effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on reducing premature fruit drop. Different concentration levels of the 2,4-D (8, 16 and 20 mg/L were applied exogenously to mature fruit trees of sweet orange (*Citrus sinensis* L.) in the 2004/2005 season. In the 2005/2006 season the 2,4-D treatments ranged from 20 to 40 mg/L concentration. There appeared a general increase in fruit drop for the month of October in all treatments but a decrease in fruit drop was observed in the fruit trees with 16 and 20 mg/L 2,4-D concentration, that is, from November through February; with the latter showing the least number of fruits that dropped throughout the execution of the experiments. The application of 20 mg/L 2,4-D significantly reduced fruit drop by more than 50% but higher concentration levels of the plant growth regulator significantly increased fruit drop. It was also evident that, small sized fruits were more susceptible to fruit drop than larger fruits. These findings suggested that, 2,4-D can be an effective tool to control fruit drop by enhancing retention, as well as improving the quality of navel oranges under dry climatic conditions.

Key words: *Citrus sinensis* (L), fruit drop, plant growth regulator, 2,4-dichlorophenoxyacetic acid.

INTRODUCTION

Citrus generally thrive well under subtropical conditions such as those of Southern Africa but the overall output is very low due to a relatively small area of production thus leading to low yields (MFDP, 2003).

The relatively small hectare-age, from which citrus fruits are produced, is largely attributed to the relative infancy of citrus production and the small number of people that are engaged in production (Seleka, 1999). This is further compounded by physiological problems associated with fruit production such as excess premature fruit drop and production of small sized fruits (Rice et al., 1990). Low and variable rainfall as well as other adverse climatic factors can also be attributed to low productivity.

Davies and Albrigo (1994) associated premature fruit

drop of many fruit crops particularly of the oranges with several factors such as high temperatures and water deficits, poor nutritional management, pests' attacks, and winds of high velocities. Fruit trees often produce profuse flowers and consequently a substantial number of fruits will later shed off as a way of reducing heavy fruits load so that the tree remains with the fruits that it can sustain. In some cases, a combination of factors rather than a single factor causes fruit drop and that makes diagnosis and remedial measures to reduce premature fruit drop, rather difficult. In other instances, plant growth regulators that are often used become antagonistic to one another when subjected to stress factors such as water stress (Mahouachi et al., 2005).

The period where fruit drop commences is believed to be at anthesis and continues until the time of harvest (Davis and Albrigo, 1994). The initial drop period involves the abscission of 'weak' fruitlets occurring until 3 - 4 weeks post anthesis. In the early stages, as the size of the

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fruit is very small, fruit drop is minimal; dropping is most tremendous in medium-sized fruits. During this period, the dropping fruits would cover the whole area under the tree (Davies, 1986).

Plant growth regulators (PGR's) are known to have a great influence on fruit drop and fruit retention in fruit trees. An imbalance of auxins, cytokinins and gibberelins for example may lead to the formation of abscission layer at the stem point and eventually fruit drop (Lahey et al., 2004; Chen et al., 2006).

The application of PGR's can re-enforce hormone balance or retard the precocious fall and the losses prior to harvest. The use of auxins prevents dropping of fruit by maintaining the cells at zone of abscission, preventing the synthesis of hydrolytic enzymes such as cellulase, which decompose the cell wall. The compound 2,4-dichlorophenoxyacetic acid (2,4-D) is regarded as one of the most effective ones in preventing fruit drop in citrus (Coggins and Hield, 1968; Coggins and Lovatt, 2004). However, the utilization of this PGR for reducing fruit drop only became common in later years in citrus producing areas such as California. Other auxin-type PGR's, that can also be utilised to prevent fruit drop include 2,4,5-trichlorophenoxypropionic acid (2,4,5-TPA) and naphthaleneacetic acid (NAA) predominantly, as well as other PGR's such as gibberellic acid (GA) (Michael et al., 1999).

In the present study we have determined the efficacy level of 2,4-D in preventing fruit drop in navel oranges under Botswana conditions. Notwithstanding the fact that, premature fruit drop may be caused by poor management of orchards as well as by other biotic factors. The findings from this study would be useful in decreasing premature fruit drop in oranges in the ever growing citrus industry in the country, thereby increasing yields.

MATERIALS AND METHODS

Experimental site description

The research was conducted under field conditions at Botswana College of Agriculture (BCA) orchard, from October 2004 to March 2006. BCA is located approximately 10 km north of Gaborone at an altitude of 24° 33' South and longitude 25° 54' East and an elevation of 994 m above sea level. The climate is semi arid with an average annual rainfall of about 538 mm (Bekker and de Wit, 1991). The studies were carried out during the summer months of October to February where maximum temperatures could be as high as 39°C or above and rainfall usually continues to March/April.

Plant selection and experimental design

Twelve (12) fully matured navel orange trees were randomly selected for use in the investigations. Trees were divided into three replicate groups basing on the four treatments that were applied.

A split plot approach was adopted for the experimental design. The main plot being the concentration of the 2,4-D applied to the trees while the subplots were the two sides of the tree selected (eastern and western side of the tree). This was done in order to eliminate the effect of the sunlight on fruit drop.

During the course of the experiment, the normal cultural practices with regard to nutrition, weed control pest and disease control were adopted.

Preparation of 2,4-D solution and sprayer calibration

A stock solution of 4.8 g/L was prepared from the concentrated solution of 2,4-D (480 g/L) and the prepared solution was refrigerated at a temperature of about 4 - 7°C.

A 6 L knapsack sprayer was calibrated, each of the 12 fruit trees was sprayed to a point of run-off. It was observed that, about 5 L was required to spray one tree to a point of run off in 10 min at an approximate pressure of 28 kg/cm².

The results from the calibration of the sprayer enabled an accurate determination of the volume of 2,4-D solution and pressure required to spray a single experimental tree.

The compound 2,4-D was applied to nine trees in various concentrations (8, 16 and 20 mg/L) with each treatment replicated thrice. The three control trees were used in the investigation had distilled water applied to them.

In order to be able to determine the threshold level of 2,4-D efficacy, a repeat experiment but with higher concentrations of 2,4-D (20, 30 and 40 mg/L) with the same control was carried out in the following season.

Each treatment was applied by spraying a whole tree evenly with 5 L of 2,4-D solution, but in the case of treatment 1 (control), distilled water was sprayed onto the trees. Trees were first foliar sprayed with the chemical in November, thereafter; application of treatment was carried out after a period of 30 days for the second and the third treatment, as according to EL-Otmani (1992).

Determination of fruit drop

Two medium sized branches were selected towards the edge of the tree branches, on opposite sides of the fruit trees. One was selected on the eastern orientation of the tree and the other on the western side. The fruits on the branches were counted using a manual counter, prior to the application of the PGR. Fruit counting resumed a month after application of treatment, on weekly interval. All fruits dropped prior to the application of the chemical were counted on weekly bases and after counting the fruits were removed from under the tree. The same practice was carried out even after the application of the treatments. Observations on fruit drop were recorded during the months of October, November, December, January and February.

Size of the fruits that dropped

The area beneath the canopy of the tree was divided into four quarters. Thereafter, two quarters from opposite sides were selected from each tree. The diameter of dropped fruits in those quarters was measured using vernier callipers and recorded once every week, that is, prior and after the application of the treatments and after treatments application.

Good quality of fruits retained

The number of good quality fruits retained on the branches and weight of the retained fruits were determined. Fruits left on the selected branches were also counted on monthly basis.

The weights of the retained fruits were measured using an electric balance. In addition, the harvested fruits were separated basing on the colours they showed at the termination of the experiment (green or greenish-yellow). The premise was that the greenish-

Table 1. Influence of 2,4-D concentration on the natural fall of navel oranges in 2004/2005.

| Concentration of 2,4-D (mg/L) | Mean number of fruit (Oct-Feb) |
|-------------------------------|--------------------------------|
| 0 (Distilled water) | 498a |
| 8 | 345b |
| 16 | 296b |
| 20 | 209c |
| Mean | 340 |
| C.V (%) | 8.15 |

Values with the same letter are not significantly different from each other at $P < 0.05$.

Table 2. Influence of 2,4-D concentrations on the number of good quality fruits retained in the 2004/2005 season.

| Concentration (mg/L) | Number of fruits retained |
|----------------------|---------------------------|
| 0 | 8c |
| 8 | 13bc |
| 16 | 16ab |
| 20 | 19a |
| Mean | 14 |
| C.V | 26.31 |

Values with the same letter are not significantly different from each other at $P < 0.05$.

yellowish fruits were closer to maturity and good quality fruits retained were those that were close to maturity at the termination of the experiments.

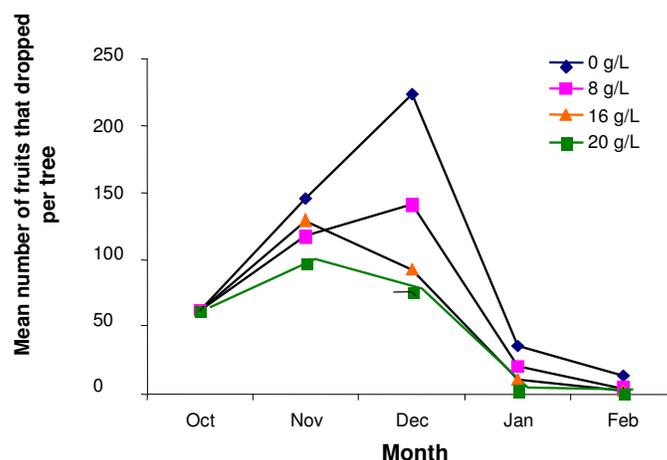
Data analysis

Data collected were subjected to analysis of variance (ANOVA) using the statistical analysis system (SAS, 2003) and the results were considered to be significant at the 0.05 level of probability. Means were separated using the Least Significant Difference (LSD).

RESULTS

The effects of the various concentrations of 2,4-D on the mean fruit drop are shown in Table 1. There were no significant differences in the number of fruits that dropped between trees treated with 8 and 16 mg/L of 2,4-D herbicide, but the control (0 mg/L) treatment had the highest significant number of fruit drop.

However, the 20 mg/L treatment had the least number of fruits dropped. This implied that, an increase in 2,4-D reduced the number of fruit fall. There were increasing trends in the number of fruit drop from the month of October in all treatments (Figure 1). Trees treated with 16 and 20 mg/L 2,4-D, however, reached the maximum fruit drop in November while those treated with 0 (control) and

**Figure 1.** Influence of 2,4-D concentration on fruit drop over time.

8 mg/L reached the maximum fruit drop on December followed by decreased in number of fruits dropped in the following months.

Fruit trees that were treated with 20 mg/L of 2,4-D exhibited the least number of fruit drop while the control showed the maximum number of fruit drop throughout the duration of the experiment.

It appeared that, on average, small sized fruits (0.1 to 0.5 cm) were more susceptible to fruit drop than the larger sized fruits (Figure 2).

There were no significant differences in the number of fruits retained on the tree treated with 16 and 20 mg/L of 2,4-D herbicide (Table 2). Also there were no significant differences on the number of fruits retained on the control trees and those treated with 8 mg/L PGR. However, there were significant differences on the number of fruits retained between the trees treated with 8 and 20 mg/L, and also between 0 and 20 mg/L. The number of fruits retained on the trees treated with 0 and 16 mg/L were significantly different. Generally the results indicate that the more the concentration of the PGR applied, the more the number of fruits retained.

There was a significant difference on the fruit weight retained between concentration 8, 16 and 20 mg/L (Table 3). Results indicate that an increase in concentration of 2,4-D increased the average weight of the retained fruits.

With regards to the colours of the retained fruits; a significant difference was observed between trees treated with 20 mg/L (2,4-D) and other treatments, but there were no significant differences among the two other concentrations applied to the trees as well as the control. There were significant differences in the number of greenish-yellow coloured fruits that were retained on a branch, between the trees that were that were treated with 16 and 20 mg/L of the chemical. The 20 mg/L treatment resulted in a significantly lower number of green fruits, higher number of greenish yellow fruits and a higher mean weight of harvested fruits per branch (Table 4).

Table 3. Influence of 2,4-D on the average weight of fruit retained.

| Concentration (mg/L) | Average fruit weight (g) |
|----------------------|--------------------------|
| 0 | 105 |
| 8 | 146 |
| 16 | 181 |
| 20 | 241 |

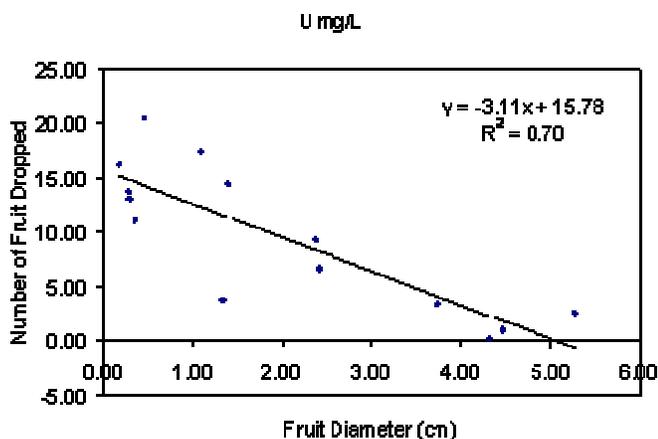


Figure 2. Fruit drop from the control versus average fruit diameter.

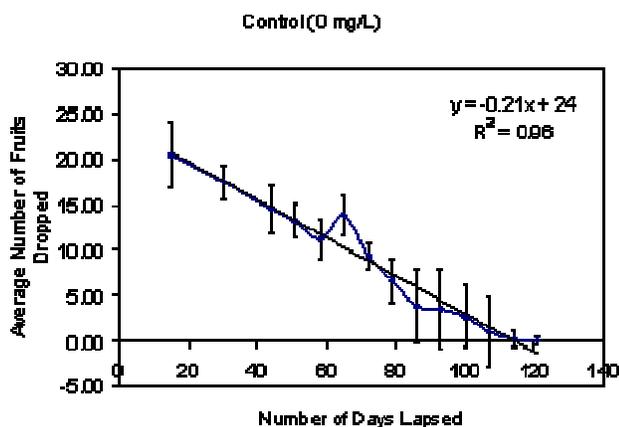


Figure 3. Fruit drop from the control application.

In the trees that were under the control (distilled water) treatment, smaller fruits of diameter of 0.1 to 1.0 cm (Figure 2) were more susceptible to fruit drop and those of 5 cm in diameter were less susceptible.

In the 2005/2006, season where higher 2,4-D concentrations were used than in the 2004/2005 season, the control (0 mg/L) showed decreasing trends in the number of fruits dropped with the graph falling at the gradient of -0.21 (Figure 3). For the first 20 days the average number

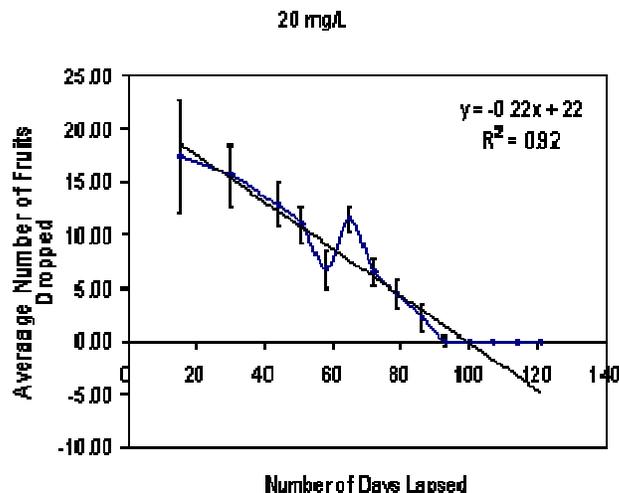


Figure 4. Fruit drop from trees treated with 20 mg/L 2,4-D.

of fruits that dropped was high and it lowered between 100th and 120th days. The graph indicates that, at around 120 days, fruit drop totally ceased to occur.

When 20 mg/L of 2,4-D was applied to the fruit trees, there was a decreasing trend in the average number of fruits that dropped, falling at the gradient of -0.22 (Figure 4). In the first 20 days the average number of fruits that dropped was high, but between the 80th and 100th days fruit drop ceased. The average number fruits that dropped were initially high until between the 20th and 60th days; thereafter, there was a decreasing trend in the number of fruits that dropped. The trend depicting fruit dropping increased after the 60th day.

At 30 mg/L of 2,4-D concentration (Figure 5) as well as 40 mg/L (Figure 6), there were decreasing trends in the average number fruits that dropped between 0 to 65 days and fruit drop generally increased after 65 days.

DISCUSSION

Influence of 2,4-D on fruit drop

Navel oranges have been shown to respond well to application of chemicals such as 2,4-D in controlling various fruit physiological and quality parameters such as fruit retention, premature dropping, fruit weight and size.

Fruit trees under the control treatment had the significantly highest number of premature fruit drop followed by those treated with 8 mg/L. The cause of this drop is probably related to competition among fruitlets for carbohydrates, water, and hormones (Lima and Davies, 1984). Davies (1986) remarked that navel oranges are more susceptible to environmental stress than other sweet oranges and observed that fruit drop of navel oranges during the summer, reduced yield by as much as 30%.

Table 4. Influence of 2,4-D on the quality parameters retained in the 2004/2005 season.

| Concentration (mg/L) | Mean Wt (Kg) | No. of green fruits | Greenish-yellow fruits |
|----------------------|--------------|---------------------|------------------------|
| 0 | 0.82d | 8a | 0c |
| 8 | 1.82c | 11a | 2c |
| 16 | 2.86b | 10a | 7b |
| 20 | 4.53a | 3b | 17a |
| Mean | 2.54 | 8 | 6.05 |
| C.V (%) | 16.57 | 41.67 | 30.15 |

Values with the same letter in a column are not significantly different from each other at $P < 0.05$.

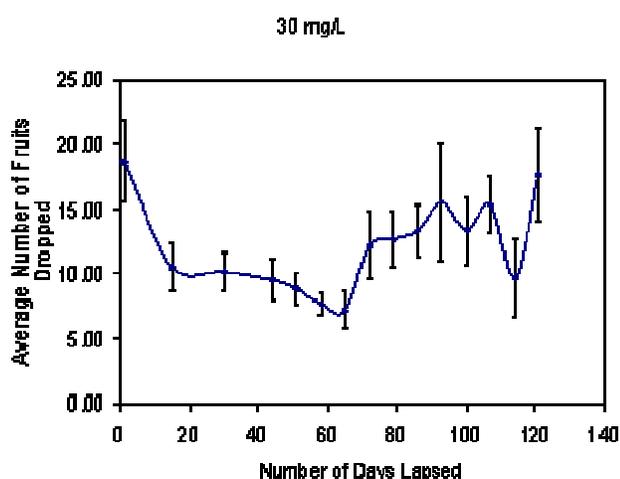


Figure 5. Fruit drop from trees treated with 2,4-D at 30 mg/L.

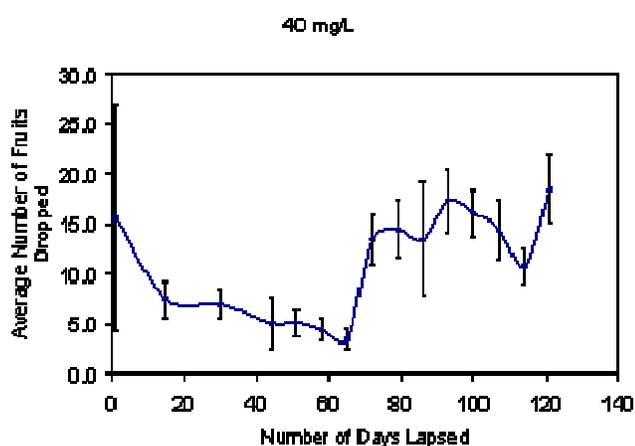


Figure 6. Fruit drop from trees treated with 2,4-D at 40 mg/L.

The lowest number of fruits that dropped as was observed in trees treated with 20 mg/L of 2,4-D, indicated that the 20 mg/L concentration of 2,4-D was the threshold level that is suitable to reduce fruit drop. It also took a

fewer days to stop fruit drop with this PGR concentration than with the higher ones. This essentially indicates that 2,4-D at 20 mg/L concentrations reduces or could retard fruit drop in a short period thereby increasing the yield of the fruit tree. Higher concentrations of 30 and 40 mg/L of 2,4-D resulted in the dropping of significantly larger numbers of fruits. It could therefore be deduced that the optimum concentration of 2,4-D to reduce fruit drop should be between 16 and 20 mg/L.

The data further suggests that, the number of fruits that drop could be reduced by at least 58%. At above the threshold level of between 20 and 30 mg/L, 2,4-D seems to exhibit herbicidal properties. This argument is ably supported by Lee (2003) who also observed that 2,4-D is an auxin-type herbicide that brings about a growth response in plants at a low concentration but at higher concentration it becomes a herbicide. Recently, Agustí et al. (2006) made quite similar observations to those in the present studies where they determined that, the percent abscission at 15 mg/L⁻¹ of 2,4-D was reduced by 50 - 75% and that the treatments had no effect on the external and internal characteristics of the fruit. Earlier on numerous other investigators including Michael et al. (1999) arrived at similar conclusions. In their studies, they concluded that fruit drop was reduced by 62% where a concentration of 16 mg/L was applied. EL-Otmani (1992) used a combination of GA₃ and 2,4-D in reducing fruit drop. He further concluded that the combination of those plant growth regulators reduced fruit drop by 78.05%. However, care should be taken when using 2,4-D and combinations; since its use can potentially affect many processes. Hield and Erickson (1962) had reported that the maximum period of effectiveness of 2,4-D in controlling fruit drop lasted three to four months from the time of treatment.

The role of auxin in abscission is complicated. It has been observed that application of auxin soon after fruit set results in an acceleration of abscission (Lee, 2003). Michael et al. (1999) remarked that the standard practice in controlling fruit drop in citrus was that, it should be applied shortly before fruit drop become a problem, but sufficiently ahead of fruit set. However, when auxin-type PGR's such as 2,4-D are applied during the mid-stages of fruit growth, abscission is delayed or prevented (García-Luis et al., 2002).

During the month of October there was a general increase in the number of fruits that dropped in all treatments. This was so because during that month, no chemicals were applied in any of the trees. The intensity of fruit drop increased during the month of November to December depending on the levels of 2,4-D concentration applied to the trees. This could be due to the unfavourable conditions such as heavy rainfall and winds of high velocity that were experienced during those months. Sinha and Mallick (1950) and Davies and Albrigo (1994) found a positive correlation between the amount of rainfall and the extent of fruit drop in oranges.

Colour of harvested fruit

There appeared an inverse relationship between 2,4-D concentration and the colouring of the fruits retained as shown by increase, in increasing concentration versus a decrease in the number of green fruits. The highest covariance (C.V.) of 41.67%, was observed in the green fruits retained. A high value of C.V may suggest that the parameter being measured may not only have been influenced by the treatment alone, but there could be other factors that came into place, such as effect of partitioning of assimilates resulting in a varying fruit response (Craighton et al., 1986).

Fruit weight

The use of 2,4-D herbicide increased the weight of the fruits retained. The results are in contrast with those of Coelho et al. (1978) and Coggins and Henning (1988), where they did not observe any effect in mass of 'Cravo' tangerines. Nonetheless, they used a different chemical, that is, GA₃ at 10, 20 and 30 mg/L concentrations. Zaragora et al. (1977), applied GA₃ in combination with 2,4-D on Washington Navel' oranges, and found no differences in the mass of retained fruits. Different plant growth regulators to 2,4-D were used in these instances. Other researchers such as García-Luis et al. (2002) and Stern et al. (2007) held the same view as in the present study. They observed 8 - 13% increase in fruit diameter due to auxin application. This could be attributed to the formation of phloem and secondary xylem which they deemed to be directly related to fruit size.

The results on effects of 2,4-D on weight of fruit retained at concentrations as in the present study needs further investigation. It would also appear though, that, application of 2,4-D accelerates fruit ripening as evidenced by the greenish-yellow colour in harvested fruits with increased 2,4-D concentration.

Influence of side orientation on the number of good quality of fruits retained

Side orientation of the tree branches had no influence on

the fruit drop or retention therefore the east/ west orientation has no effect on either the fall or retention of the fruit. Any quarter of the tree can be used in such studies without influence of the rise or setting of the sun.

Substitutes of 2,4-D

Although this chemical may be inhibitory to plant growth, information on the absorption and excretion of 2,4-D in several species (including man) indicates that the compound is rapidly excreted unchanged and that it is not stored in the tissues of mammals (FAO/WHO, 1972). Small amounts are therefore safe to use in food since the acceptable daily intake for man is 0 - 0.3 mg/kg. A dosage of 15 mg/kg was found to be non-toxic in humans, while ingestion of a high dosage of about 90 mg/kg was fatal (Moore, 1989). The European Union (EU) has ceased to register 2,4-D isopropyl ester for use to reduce fruit drop, instead 2,4-DP (2 ethylhexyl ester) has been registered as a replacement for 2,4-D (Augustí, 2006).

Anthony and Coggins (2001) presented NAA and 3, 5, 6-TPA as potential chemicals for controlling fruit drop in citrus under California conditions. Stover (2000) suggested the use of GA₃ and NAA, and these have also been used for pre-harvest fruit drop control in Californian citrus crops. Potentially GA₃ could not only be used to reduce early fruit abscission in citrus but also to delay senescence (Hershely, 2001).

Although NAA has been used to reduce abscission of fruit, Marini et al. (1993), cautions that repeated application of more than the single application of the compound, delays fruit abscission.

In other fruits such as apples, Bangerth (1978) and Boller et al. (1979) determined that multiple application of aminotheoxyvinylglycine (AVG) a natural occurring hormone, retarded pre-harvest drop by inhibiting ethylene biosynthesis. In countries where legislation does not prohibit the use of 2,4-D it may take a long time to use its substitutes.

Conclusions

This study has shown that 2,4-D at concentration of 16 to 20 mg/L has ability to reduce fruit drop of navel oranges significantly when compared to the lower concentration levels. The fruits are also larger. The higher concentration level the more the fruits are retained on the tree up to the threshold level, thereafter 2,4-D exhibits herbicidal properties by increasing fruit drop. The 2,4-D chemical could thus be used to increase fruit yield under conditions that favour fruit drop such as extreme temperatures as well as water deficits such as those that are prevalent in Botswana.

The application of 2,4-D is more effective at the earlier stages of fruit development since navel oranges were shown to be more susceptible to fruit drop when small in size.

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