

## Abscission of Control Pollinated Coconut Fruits: Preliminary Investigations into the Possible Role of Ethylene

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### Abstract

Recent advances in the spread of the Cape St Paul Wilt disease (CSPWD) necessitate a more rapid development of tolerant coconut types. Control pollination is the main method for producing legitimate coconut seeds for such breeding purposes. However, the process of bagging causes severe nut drop or abscission. Many scientists have implicated hormones as the primary regulators of organ abscission; with ethylene being particularly implicated. Unlike other plant hormones, which are mainly produced in other parts and transported to influence target organs, the production of ethylene could be initiated within the same organ, such as the fruit. This study was therefore undertaken to investigate the possible involvement of ethylene in abscission after control pollination. Pollen from Vanuatu Tall (VTT) variety was used to cross emasculated Malayan Yellow Dwarf (MYD) inflorescence after isolation with bags. Trials were laid out in Completely Randomised and Split Plot designs, with specified replications. Two ethylene inhibitors, namely aminoethoxyvinylglycine (AVG) and silver thiosulphate (STS) were used. Five concentrations of AVG ranging from 2mg/l to 30mg/l were applied to inflorescences during and after bagging. AVG concentrations higher than 2 mg/l resulted in significant reduction in abscission. Fruit yields up to five times that of ordinarily bagged treatments without AVG were recorded. Four concentrations of STS ranging between 0.5mM – 10mM applied in similar manner, however, did not result in any significant reduction in abscission. The results from AVG application strongly suggest the involvement of ethylene in the abscission of control pollinated coconut fruits.

**Keywords:** Control Pollination, AVG, Bagging, Abscission, Ethylene

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## Introduction

Over two decades of intensive field screening in Ghana have led to the identification of some coconut types, which are resistant or less susceptible to the Cape St Paul Wilt disease (CSPWD). These include the Sri Lanka Green Dwarf variety (SGD), which has been ranked as resistant and the Vanuatu Tall variety (VTT), which has so far exhibited appreciable levels of tolerance to the CSPWD. The hybrid between the Malayan Yellow Dwarf variety (MYD) and the VTT has also shown moderate tolerance (Dery *et al.*, 2005; Nipah, 2000). Some of these promising types, however, have undesirable traits that make them uneconomical for release as commercial varieties. Unlike the local West African Tall (WAT) variety, the SGD is very susceptible to attack by insects and does not tolerate harsh environmental conditions. Also, resistance could break down with time; therefore, it is imperative that these modest advances be exploited to develop improved varieties and hybrids to ensure lasting resistance and high productivity. This requires reliable breeding techniques, which eliminate errors that may only be detected after many years.

Clonal propagation or tissue culture, which has proved very useful in many crops, has not been very successful with coconut. Since the 1960s, many research projects have been directed towards developing a method of clonal propagation, but success has been very limited (Branton and Blake, 1983; Pannetier and Buffard-Morel, 1986; Thanh-Tuyen, 1990; Blake, 1990 & 1995; Bourdeix, 1999). But the need to reduce the rate of spread of the CSPWD is so urgent that it is imperative to concentrate on other techniques to make progress.

The main method that has been used in improvement programmes is the control pollination. This involves bagging emasculated inflorescence of selected palms and introducing artificially processed pollen from individual palms with desired traits. However, in coconuts the control pollination process severely reduces yields through a combination of low fruit set and high abscission rates. This makes the process very expensive and time consuming, and creates

constraints to progeny assessment. It has therefore been a major objective of the national coconut breeding programme, to identify the causes of abscission in control pollinated coconut fruits.

Earlier work by Nipah *et al.* (2009) ruled out relative humidity, light intensity and temperature around the inflorescence during the bagging period as the main causes of the high abscission rates. Many authors have however, claimed that abscission of organs may be primarily regulated by hormones (Tamas *et al.*, 1979; Gianfagna, 1987; Van Meeteren and Van Gelder, 1995; Aloni *et al.*, 1996; Kimball, 2006). Ethylene, which is a simple unsaturated hydrocarbon ( $H_2C=CH_2$ ) produced in all higher plants (Roper, 2005), is particularly considered by many to be the natural regulator of abscission (Reid, 1985; Sexton *et al.*, 1985; 1995; Osborne, 1991). Other plant hormones are mainly produced in other parts of the plant and transported to influence the development of target organs. The production of ethylene, which is a gaseous hormone, could however be initiated within the same organ such as the seed. Studies have confirmed seeds as main sources of ethylene production in many crops. A transitory climacteric (peak production) of ethylene is detectable prior to senescence in oilseed rape pods, much of which is attributable to the seeds (Meakin and Roberts, 1990). Ethylene is also involved in the senescence caused by other hormones in some plants. The senescence caused by ABA is mediated through ethylene in carnation flowers and certain cultivars of miniature roses (Ronen and Mayak, 1981; Muller *et al.*, 1999; Hunter *et al.*, 2004). Therefore if hormonal factors are involved in the high levels of abscission experienced in control pollinated coconut fruits, ethylene is most likely to be detected.

In the ethylene production pathway, ACC synthase is the most crucial enzyme. The enzyme is induced by several factors including fruit ripening, wounding, drought, flooding, etc. (Saupe, 2007). Cutting off the spathe and stripping the male flowers from the spikelets to prepare the coconut inflorescence for control pollination create wounds which may induce

ACC synthase and lead to the production of higher levels of ethylene. Furthermore the isolation bag is likely to limit air circulation to some extent within the bag. This will tend to restrict the escape of any ethylene released from the inflorescence. Therefore, not only could there be increased ethylene concentration, but also there will be a corresponding increase in the period to which the inflorescence is exposed to the hormone.

Plant growth regulators (PGRs) have been used with success to control the effects of hormones in plants. These products could mimic naturally occurring plant hormones or block their production (Roper, 2005). Aminoethoxyvinylglycine (AVG) blocks the action of ACC synthase (Saupe, 2007), and therefore could prevent the production of ethylene. AVG has been used as spray solution on a number of plants, and has registered great successes in apples and pears. Silver ions ( $Ag^+$ ),  $CO_2$  and  $KMnO_4$  can also inhibit ethylene actions. These bind to ethylene receptors or otherwise interfere with the mechanism of ethylene action (Saupe, 2007). Petal abscission in red raspberry (*Rubus idaeus* L.) flowers was effectively retarded by silver thiosulphate (Burdon and Sexton, 1993).

The effectiveness of a PGR application is determined in part by dosage and timing (Roper, 2005). The objective of this study therefore was to study the effects of two ethylene inhibitors, namely aminoethoxyvinylglycine (AVG) and silver thiosulphate (STS) to determine whether the control pollination process induces the production of ethylene.

### Materials and methods

#### a) Field experimentation

Field experiments were conducted between February 2005 and September 2005. Trials were laid out in completely randomised and split plot designs, with specified numbers of replications. Standard pollination methods explained by Nipah *et al* (2009) were adopted.

#### b) Effects of wounding and bagging on fruit abscission

It was suspected that wounds created by removal of the spathes and male flowers, or the bagging process, or a combination of these actions cause the high level of abscission. To investigate this, experiments were designed to progressively increase the level of wounding among bagged and non-bagged inflorescences, using treatments which did not require the use of processed pollen. Five treatments involving self pollinated and open pollinated inflorescences were laid out in completely randomised design with 6 replications, using Malayan Yellow Dwarf (MYD) mother palms at Bonsaso. The treatments were:

(T1) No wounding and no bagging i.e. naturally pollinated palms;

(T2) Wounding by spathe removal only, and without bagging;

(T3) Wounding by spathe and male flower removal, but no bagging, i.e. open pollinated with no possibility for selfing;

(T4) no wounding but bagged. Newly opened inflorescences were simply bagged with the opened spathes and male flowers still intact; and

(T5) wounding by spathe removal only followed by bagging.

A treatment involving wounding by spathe and male flower removal followed by bagging was not included because that would require the use of processed pollen. Yields, represented by the percentage of fruits/female flowers, 6 months after pollination were analysed.

#### c) Effect of aminoethoxyvinylglycine (AVG) on fruit abscission

AVG inhibits ethylene biosynthesis by blocking the action of ACC synthase (Saupe, 2007). The application of the right concentration of the product at the right time was therefore expected to reduce fruit abscission, should ethylene be involved. This study was aimed at determining whether any particular concentration of AVG applied at a certain time

during the early developmental stages of the fruit could reduce the level of abscission; and consequently prove the involvement of ethylene in the process.

5 mg of AVG [active ingredient: S-trans-2-amino-4-(2-aminoethoxy)-3-butenoic acid] in powder form was used to prepare a 30 mg/l stock solution, by diluting the product in 167 ml of water. From the 30 mg/l stock solution, five concentrations measuring 70 – 80 ml were derived; namely 30 mg/l, 20 mg/l, 10 mg/l, 5 mg/l and 2 mg/l, as well as ordinary sterile water representing 0 mg/l. Each treatment was applied with the aid of spray guns, to three different inflorescences as sub treatments on: The day of emasculation and bagging (DE/B), the day of bag removal (DBR) and 7 days (1 week) after bag removal (WABR). The trial was set up in March 2005 at Bonsaso on MYD mother palms, in a split plot design with three replications. About 8 ml of AVG solution was sprayed around each inflorescence in a single application.

#### **d) Effect of silver thiosulphate (STS) on fruit abscission**

Silver thiosulphate (STS) is an ethylene action inhibitor, i.e. it blocks ethylene action after its production (Sisler *et al.*, 1983; Veen, 1986; Sexton *et al.*, 1995). This trial was therefore set up to determine whether the application of any particular concentration of STS at a certain time during the control pollination process could reduce the levels of fruit dropping; and thereby confirm that ethylene was a major cause of fruit abscission in coconut.

STS was prepared from stock solutions of sodium thiosulphate and silver nitrate.

0.1 M sodium thiosulphate solution was prepared by dissolving 1.58 g of sodium thiosulphate into 100 ml of water.

0.1 M silver nitrate stock solution was prepared by dissolving 1.7 g silver nitrate into 100 ml of water.

The two stock solutions were kept in the dark until needed to prepare STS. In general, STS is prepared with a molar ratio between silver and thiosulphate of 1:4, respectively (Sigma-Aldrich,

2005). When required, the stock solutions were taken to the field in dark brown bottles covered with aluminium foil, and used to prepare a fresh solution of STS for immediate application. 20 ml of 0.1 M silver nitrate stock solution was slowly poured into 80 ml of 0.1 M sodium thiosulphate stock solution while shaking the bottle gently, to prepare 100 ml of a 0.02 M stock solution of STS. In this way, nearly all the silver present in the solution was in the form of  $[Ag(S_2O_3)_2]^{3-}$ , the active complex for ethylene effect inhibition (Sigma-Aldrich, 2005).

An initial trial was set up in March 2005. Four concentrations of STS solution were derived as main treatments, by diluting 0.02 M stock solution with appropriate amounts of distilled water to obtain 0.5 mM, 1.0 mM, 5.0 mM and 10 mM of STS. Ordinary sterile water was also used to represent 0 mM concentration. Each treatment was applied to four MYD inflorescences on different occasions during the control pollination process as sub treatments, in a split plot design with four replications at Bonsaso. With the aid of spray guns, about 25 ml of each concentration was sprayed in a single application onto a control pollinated inflorescence on: The day of emasculation/bagging (DE/B), 14 days after bagging (DAB), the day of bag removal (DBR) and two weeks after bag removal (2 WABR).

In April 2005, following a preliminary assessment of the initial experiment, an additional trial was set up to further assess the effects of multiple applications of each treatment on an inflorescence, as well as the effect of a higher concentration. Four STS concentrations of 1.0 mM, 5.0 mM, 10 mM and 20 mM, as well as 0 mM were applied as main treatments. Each main treatment was applied to two inflorescences in two different ways (sub treatments):

Three applications: Treatments were applied initially on 14 DAB then repeated on DBR, and finally 1 WABR.

Eight applications: Treatments were first applied on 14 DAB and repeated at 2-day intervals till 1 WABR.

## Results

### a. Effects of wounding and bagging

The results indicated that removing only the spathes did not significantly alter the yield (25.9%), but removing both spathes and male flowers resulted in average yield of 23.7%, which was significantly different from the yields of the naturally pollinated inflorescence (30.1%). These differences, though significant were relatively marginal in comparison with the differences between bagged and non-bagged treatments, which were highly significant. Bagging resulted in yields below 15.0% (figure 1). The results implied that though the wounds created on the inflorescence in preparation for bagging did contribute to abscission, it was mainly the bagging that triggered the high rates of abscission in controlled pollinated coconuts.

### b. Effect of AVG

The results showed that with the exception of 2 mg/l AVG concentration, which did not have any significant effect on yield; the application of higher concentrations of AVG on the inflorescence resulted in significant increases in fruit yields in at least one of the three times of application. There were also significant interactions between AVG concentration and time of application. As shown in figure 2, when the chemical was applied on DE/B, significant increases in yield were recorded among inflorescences treated with 10 mg/l, 20 mg/l and 30 mg/l AVG.

Applications made on DBR also resulted in quite similar levels of increase in the fruit yields of those treated with 20 mg/l and 30 mg/l AVG. Surprisingly, while 10 mg/l AVG applied on DBR resulted in only marginal increase in yield (the increase was not statistically significant), 5 mg/l AVG applied on the same day resulted in the highest increase in yield among all the bagged treatments (figure 2). The average yield of 20.9% fruit/flowers resulting from 5 mg/l AVG applied on this occasion was more than five times the yield of the normal control pollinated inflorescence treated with 0 mg/l AVG (3.4% fruit/flowers), and also represented more than half the open pollinated average yield of 38%

(figure 3). Among others, significant interaction was also established between 5 mg/l AVG and application at DBR. It is quite surprising that the application of 10 mg/l on DBR was not effective. This is because other concentrations applied on DE/B, which resulted in significant increases in yield were still effective when applied on DBR (i.e. 20 and 30 mg/l). Also 5 mg/l concentration, which was not effective on DE/B was now having such a tremendous effect on yield. It is even more surprising in considering the fact that when 10 mg/l was applied one week after bag removal (WABR), it again resulted in significant yield increases. The result of the application of 10 mg/l at DBR may therefore have been erroneous. Applications made on WABR also resulted in significant yield increase by 30 mg/l concentration; but 5 mg/l and 20 mg/l concentrations had no effect on fruit yield.

### c. Effect of STS

In both experiments, the treatments did not result in any significant reduction in abscission, as indicated in figure 4 and 5. In other words, regardless of when STS was applied, and the number of times the applications were carried out on an inflorescence, the concentrations used in this experiment did not result in any significant reduction in the levels of abscission.

## Discussion

Mechanical wounding is known to cause numerous physiological and molecular reactions in plants, including abscission (Kostenyuk and Burns, 2004). Some crop scientists believe that artificial pollination does not result in abscission in oil palm because the flowers are either wholly female or male (dioecious); which does not necessitate emasculation before bagging the female flowers. In this experiment, the effects of the wounds created on the inflorescence through spathe and male flower removal, resulted in only marginal increases in abscission. Bagging was the main factor causing the high levels of abscission.

This study implicated ethylene as being involved in fruit abscission. It was not possible

Figure 1. The effects of wounding and bagging on the yield of controlled pollinated inflorescence

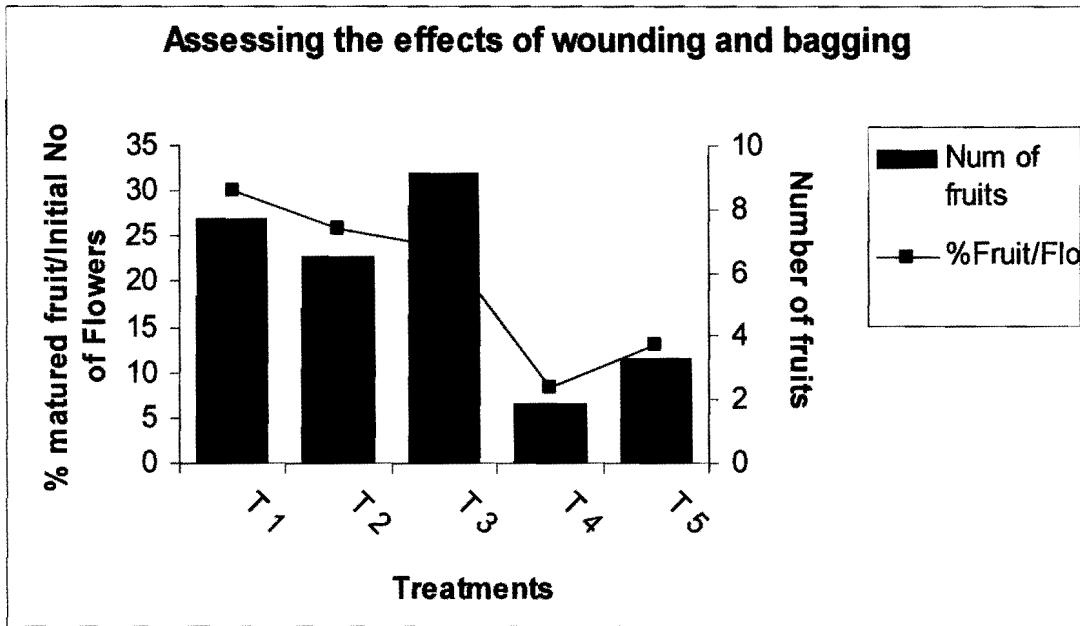
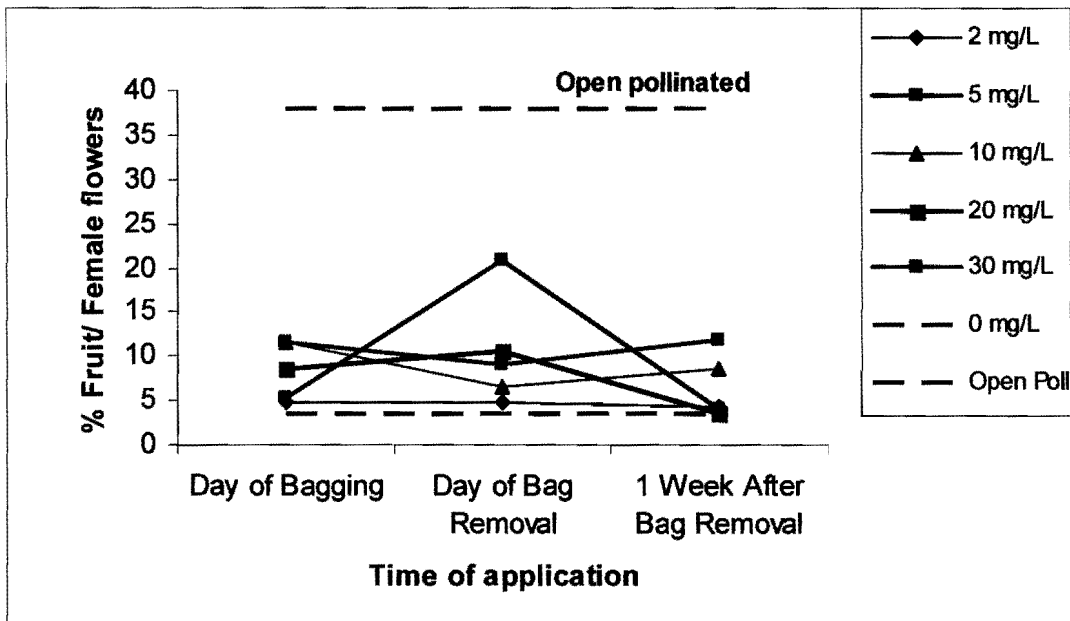
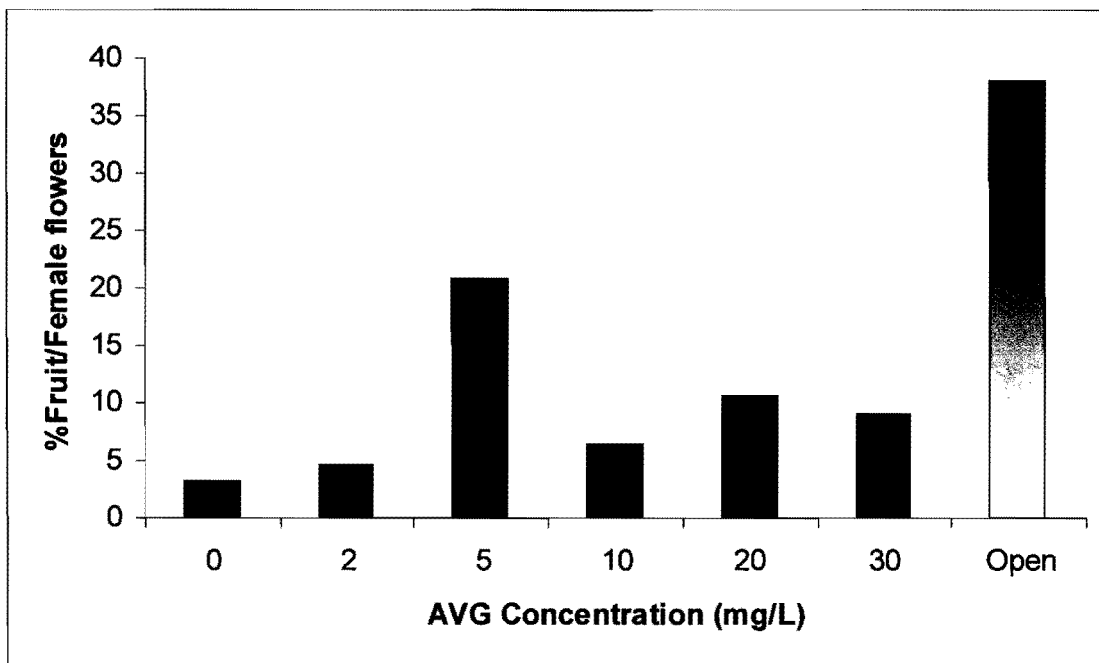


Figure 2. Effect of AVG concentration and time of application on the yield of control pollinated inflorescence



**Figure 3. The effect of applying different AVG concentrations at DBR on the yield of bagged coconut inflorescences**



**Figure 4. The effect of STS concentration and time of application on the yield of controlled pollinated coconut inflorescence**

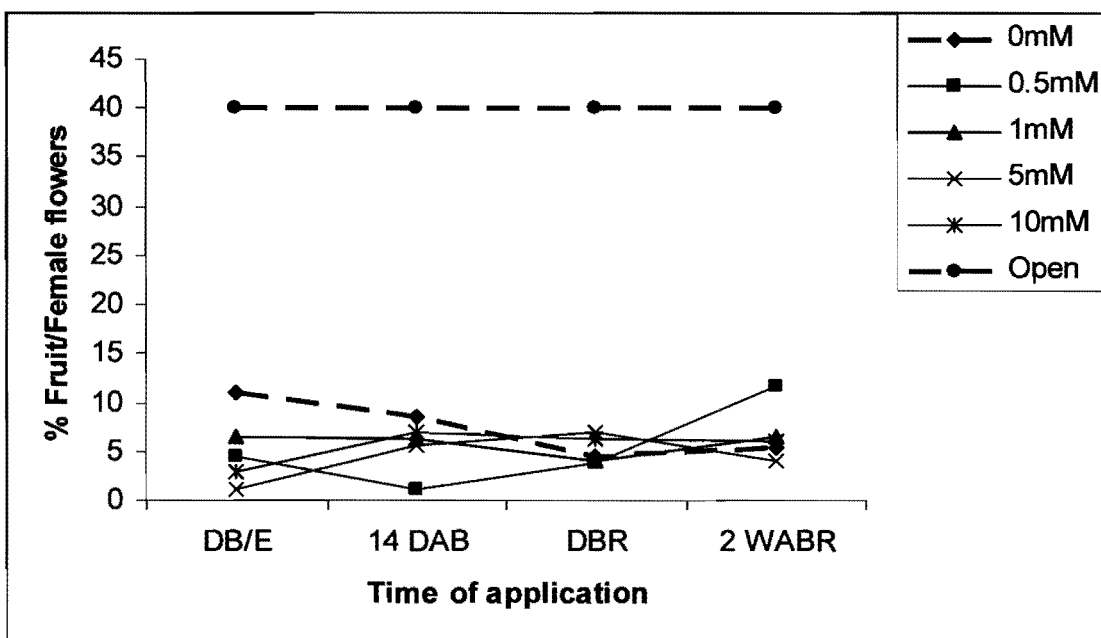
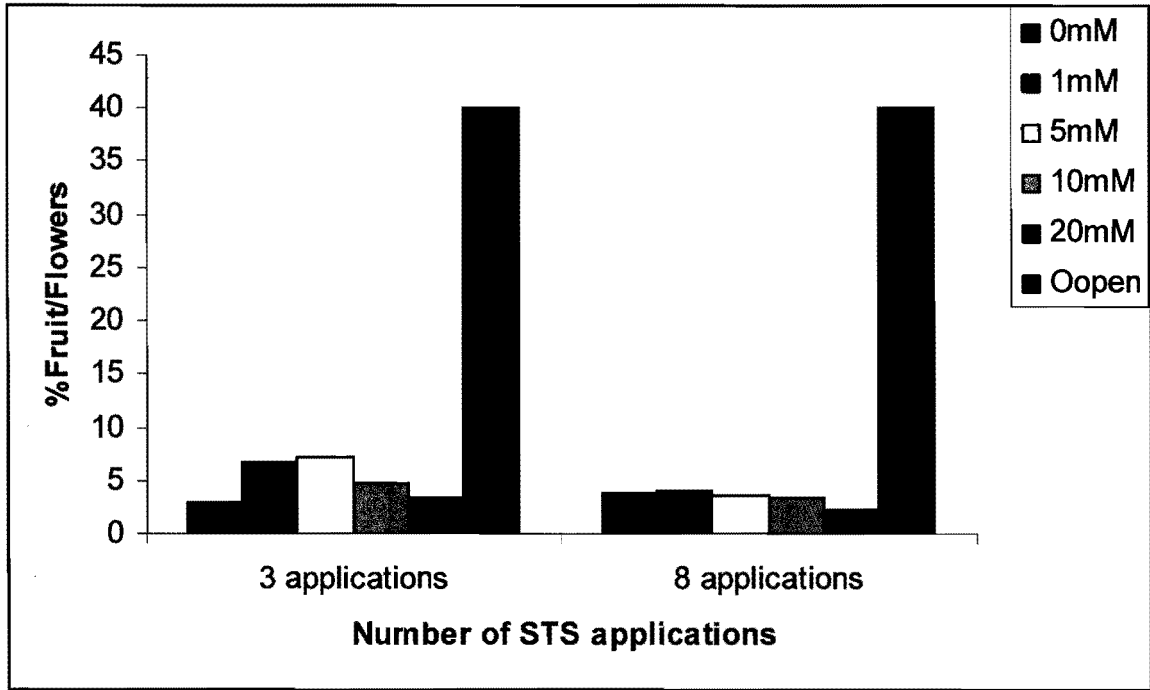


Figure 5. Effect of multiple STS application on the yield of controlled pollinated inflorescence



to measure either the total production of ethylene or the residual levels in the tissues of inflorescences to make any meaningful comparisons among the different treatments. It is however expected that with ethylene being a gaseous hormone, any excess production would simply diffuse into the atmosphere in non-bagged inflorescences, and therefore fail to affect abscission rates. In assisted pollination for instance, inflorescences are emasculated by simply cutting off the portion of the spikelets above the female flowers, which contain the male flowers, to save time due to the usual high numbers emasculated. Though this process creates a massive number of wounds along the length of the inflorescence, field experience has shown that it may not increase the level of abscission in assisted pollination. In control pollination however, ethylene produced and released within the isolation bag may be restricted from escaping freely into the atmosphere; therefore it remains concentrated around the inflorescence and for longer periods. ACC synthase is known to be induced in

response to ethylene itself (Saupe, 2007). Therefore, as it is generally said; "one bad apple spoils the whole barrel", situations that trigger the production of even small volumes of ethylene within enclosed environments, result in a cycle that induces the production of ACC synthase, which results in the production of more ethylene. The restriction imposed by the bags to the escape of any ethylene produced is therefore likely to be a major factor in the accelerated rate of abscission in bagged inflorescence.

Yields, represented by the percentage of fruits/female flowers, 6 months after pollination show that AVG concentrations above 2 mg/l reduce the level of abscission at certain times of application and clearly proves the involvement of ethylene in the process. The chemical successfully interrupted the ethylene production process; and thereby reduced the amount of the hormone released, which consequently led to lower abscission rates. In analysing the results, the main concern was to identify when ethylene production was likely to reach the minimum levels required to initiate the cell degradation

process, which leads eventually to abscission. This will help determine when it might be best to apply the chemical to achieve its optimum effect on yield. Abscission is not an instantaneous action; it is an active developmental process, which progresses at the abscission zone of the fruit peduncle, as target cells within the zone become increasingly more sensitive with increase in ethylene production (Cronje *et al.*, 2005). In this study, the highest rates of fruit drop always occurred within the first two months after bag removal, and were more pronounced between one and four weeks after bag removal. Developing fruits, which had not abscised 5 WABR, stood a good chance of developing into matured fruits. Though it was not quite clear from the results when the required level of ethylene production was achieved during the isolation process, some inferences could be made: Among the four concentrations, which had effects on abscission, solutions with concentrations above 5 mg/l AVG were effective when applied on the day of emasculation/bagging (DE/B). Applications made on the day of bag removal (DBR), further resulted in a highly significant effect of 5 mg/l AVG concentration, together with the other higher concentrations, except 10 mg/l. Assuming the ethylene production process does not start immediately after bagging, the concentration of the active ingredient of AVG applied on DE/B and absorbed into the plant tissues would be expected to reduce over time due to translocation and other processes. By the time ethylene production is due, there must however be enough active ingredients to interfere effectively with the process. What is therefore likely to have happened was that tissues of the inflorescences sprayed with 5 mg/l AVG at DE/B must have lost some active ingredient, and consequently had less than the required levels to effectively block the ethylene production process by the time it began. This could also explain why higher concentrations of AVG applied on the same occasion successfully influenced abscission; because there must have been enough active ingredients to do so. In line with this interpretation, the concentration of ethylene must have increased gradually and reached levels capable of influencing abscission rather closer to

DBR; explaining why 5 mg/l AVG applied on that occasion could have such a tremendous effect on the yield.

In spite of the clear effect of AVG on yield, the fact that some concentrations (10 mg/l and 30 mg/l) were still effective, in reducing abscission when applied one week after the bags had been removed (WABR), was however surprising. This is because, as explained in the introduction, AVG only prevents ethylene production and does not affect already produced or residual concentrations of the gas. Therefore treatments applied 1 WABR, which turned out to be effective in increasing yield, could only have restricted the production of fresh ethylene. But at this stage, when the bags have been removed, freshly produced ethylene would be expected to diffuse into the atmosphere and not have much effect on yield, as it was observed in non-bagged inflorescence. The effectiveness of late applications in reducing abscission does however give some indications that, unlike in other crops, AVG application on coconut inflorescence may have immediate impact on abscission. On apple fruits, AVG required 10 – 14 days to retard fruit drop (Greene, 2005, 2006). If such late applications of AVG required that long to start having an impact on yield, most developing fruits would have abscised before the effects become relevant.

A wide range of concentrations of AVG have been used on different plants under varying circumstances to influence crop yields. In 'McIntosh' apples (*Malus domestica* S. McIntosh), quite high concentrations ranging between 75 and 225 mg/l AVG were used in studying the effects of the product on pre-harvest fruit drop. Increasing AVG concentrations linearly reduced internal ethylene concentration in the apples, and consequently resulted in higher concentrations more effectively reducing fruit drop at maturity than lower concentrations (Schupp and Greene, 2004). Rath *et al.* (2006) also treated Kotgetsu apples (*Malus domestica* L. Borkh.) with 125 ppm of AVG and had remarkable results: AVG applied to whole trees and fruits decreased ethylene production and reduced fruit drop from an average of 58.9% to between 42.5% and, as low as, 10.4%. Also in

tomato (*Lycopersicon esculentum* Mill., cv. Castlemart), the application of 20 µl of 0.1, 3.0, and 10 mM AVG to the tissues of the locular surface of excised pericarp discs of the plant, resulted in decreases in ethylene production by 57%, 73%, and 89%, respectively (Saltveit, 2005). In this study, the highest concentration of 30 mg/l was consistently effective in all three application times; and 20 mg/l was effective on DE/B and DBR. It is important to note however that AVG is an expensive product; and therefore any consideration of its use in coconut breeding programmes must consider a balance between cost and benefits. Priority must therefore be placed on the possible use of the product at lowest possible concentration to achieve the maximum effect. The high yields resulting from the application of 5 mg/l AVG on DBR, is therefore an exciting observation, which requires further investigations. The significant interaction of that concentration with application at DBR implies that when AVG of 5 mg/l concentration is to be applied, its effect will be optimised when applied on the day of bag removal. In an experiment to study ethylene biosynthesis in oil seed rape (*Brassica napus* L.) pods in relation to pod shatter, Child *et al.* (1998) used a rate of 500 mg/l, but indicated that this concentration, which was chosen on the basis of an earlier experiment by Kushad and Poovaiah (1984), was approximately 100 times that required to inhibit ethylene-induced petiole abscission in bean explants; implying 5 mg/l as the required concentration. The authors explained that this high rate was used in order to reduce ethylene significantly.

In spite of this clear evidence of the involvement of ethylene in the abscission of control pollinated coconut fruits, STS could neither prevent nor reduce the high levels of abscission. It was not clear why this was the case; however it is possible under certain conditions for the ability of the silver ion to prevent ethylene-induced abscission to be lost. Ror (1987) reported that the ability of silver ions to prevent leaf senescence was lost in the dark in both whole seedlings and rootless, blade explants of *Vigna radiata* when either ethylene or ethephon (an ethylene-releasing compound)

were used as defoliating agents. Loss of silver activity in the dark was also observed in bladed explants of *Phaseolus vulgaris* and *Glycine max.* However, while reduced light intensity within the isolation bags may be related to applications of STS made while the bags were still in place, this phenomenon does not explain why applications made on DBR and 2 WABR, at which time the bags had been removed and therefore there was no problem with light intensity, still had no effect on abscission. The uncertainties of the results from the STS trial however do not negate the evidence provided by the use of AVG, which suggests the involvement of ethylene in the abscission process.

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