Effects of Propagation Media and Branch Orientation on Rooting of Guava (*Psidium guajava L)* Shoots Propagated by Air Layering in a Sub-tropical Environment.

ABSTRACT

Guava (*Psidium guajava* L*)* is a tropical fruit rich in vitamins and beneficial phytochemicals and thus often referred to as a super fruit. Despite the potential nutritional benefits of guava fruit, it has received little research to facilitate its wide-scale production in the Kingdom of Eswatini. This study was conducted with the aim of determining the effects of media and branch orientation on rooting of guava branches propagated by air layering. Media treatments used included vermiculite, compost, top soil, and media mix which was a mixture of top soil, pine sawdust and sand. Branches that were oriented towards the North East to South East and South West to North West directions in relation to the sun were selected for air layering. The experiment was a factorial arrangement laid out in a split plot design where there were 5 replications per treatment. Vermiculite wrapped branches showed the best adventitious root formation in relation to root length, root volume, root mass, rooting percentage and root number in comparison to other media treatments. North East to South East oriented branches produced superior adventitious root development than North West to South West orientated branches. Wrapping branches with vermiculite in combination with the selection of North East to South East oriented branches enhanced adventitious root development in air layered guava branches. It is recommended that farmers who wish to produce guavas of desired quality by air layering may select branches with the greatest exposure to the sun in the North East to South East directions using vermiculite or alternatively compost in the absence of vermiculite as propagation media.

Keywords: Guava. . Vegetative propagation. Air layering. Rooting

1. INTRODUCTION.

Guavas are plants in the Myrtaceae family, genus *Psidium*, which contains about 100 species of tropical shrubs and small trees. The most frequently eaten species, and the one often simply referred to as "the guava", is the Apple Guava (*Psidium guajava*). Guava plants have tough dark leaves that are opposite, simple, elliptic to ovate and 5–15 centimetres (2.0–5.9 in) long [1]. The flowers are white, with five petals and numerous stamens. Guava fruits are usually 4 to 12 centimeters long, are round or oval depending on the species [2]. The outer skin of guavas may be rough, often with a bitter taste, or soft and sweet. Varying between species, the skin can be of any thickness, usually green before maturity, but becomes yellow, maroon, or green when ripe [3].

Asexual propagation involves taking a part of one parent plant and causing it to regenerate itself into a new plant. The resulting new plant is genetically identical to its parent. Asexual propagation involves the vegetative parts of a plant: stems, roots, or leaves [4]. Advantages of asexual propagation are that it may be easier and faster in woody perennials such as guava, it may be the only way to perpetuate some cultivars and it bypasses the juvenile characteristics of certain species [5].

As guava will not breed true to type from seed, trees for fruit production are usually vegetatively propagated by air layering or budding. Air layering of guava trees should be done in full sun and trees should be away from buildings to prevent shading [4; 6; 7].

The process of removing a section of the trunk of a tree to create another tree is known as air layering. Layering is a method of vegetative propagation in which a branch is induced to grow roots before it is separated from the parent plant [8]. Before the branch is removed it is girdled and then sealed with plastic or other media, and the girdled section is allowed to root. After rooting the branch is removed from the tree [4; 9].

Adventitious roots are roots that arise at sites other than their usual sites such as roots originating on stems or leaves [10,11; 4]. Parrota [12] offered a more technical definition, reporting that adventitious roots arise from "buds in parenchyma tissue not directly associated with apical meristems and in places not dictated by their normal phyllotactic pattern." Bita et al. [13] lands somewhere between the previous two definitions, reporting that adventitious roots are roots that arise on parts of the plant not originating from the embryonic root; that is, the roots arise on parts of the shoot. In plant propagation, roots that form on stem or leaf cuttings are adventitious, as well as those generated from air layering and tissue culture [14].

Guava is on the priority list of the Taiwan Technical Mission (ICDF) Fruit Tree Project in the Kingdom of Eswatini. The country is faced with problems of food and nutritional insecurity and fruit production needs to be increased to partially address this problem. There is little information on the appropriate asexual production techniques (air layering) to produce guavas on a large scale that can be of the required standard quality. Asexual propagation enables reproduction of the qualities desired by the consumer. Seed propagated guava plants tend to yield fruits of variable size and quality. This study sought to provide a guide to growers who are desirous of producing guavas of acceptable quality through air layering.

2. MATERIALS AND METHODS.

2.1 Site description: The experiment was carried out in the orchard of the Department of Agricultural Research and Specialist Services at the Malkerns Research Station, in the Kingdom of Eswatini (Swaziland). The average summer maximum temperature is 27°C and 15°C in winter. The area is found in the Middleveld of Eswatini, 21°34'S and 31°12'E at an altitude of 750 m above sea level. The annual precipitation is about 800 mm, with most rainfall occurring between October and April. The soils are mostly sandy loam [15].

2.2 Soil Analysis: Soil samples were taken from the experimental site using the zigzag method, four point determinations [16]. The samples were taken to the Department of Agricultural Research and Specialist Services at Malkerns, soil testing unit laboratory, for analysis of phosphorus, potassium, exchangeable acidity and pH. Similar analysis were performed on the vermiculite and compost used in this experiment in order to help in subsequent interpretation of results.

2.3 Experimental Design: Eight trees were subjected to the air layering technique. Air layering was performed on sixty four branches. Thirty two branches were facing the South West to South East side and another thirty two branches were facing the North east to North West side and selection was in relation to the positioning of the sun. Propagation procedure was carried out on the 19th of October 2013.The branches were factorially arranged with growing media and branch selection as factors and laid out in split-plot design with five replications. The experiment was carried out for 12 weeks and data was collected at the end of the rooting period.

2.4 Air Layering Procedure: Sorensen [5] reported that the process of air layering is a four step process that starts by choosing a suitable branch and followed by gathering the necessary materials. After the material has been gathered, girdling of the selected branch is done, followed by a waiting period of a minimum of 12 weeks for rooting to occur and finally harvesting and transplanting of rooted branches thus;

Step 1: Choosing a suitable branch

The first step was to select appropriate branches. A suitable branch is usually no smaller than pencil thickness and not much larger than finger thickness. Side branches were cut. Air layering was done in the spring or early summer when the plant was actively growing.

Step 2: Gathering the materials

The next step was to gather the necessary materials. Materials used were;

Media namely vermiculite, pre-mixed media, compost and top soil

Other materials included:

1) Clear plastic wrap
2) Twine
3) A sharp knife or razor blade
4) Seradix (Bayer, Pretoria, South Africa) rooting hormone namely Seradix no3 for woody perennials such as guava for purposes of this study.

Step 3: Girdling procedure.

A section of bark around the stem was removed, a process known as girdling. Cutting off the bark was done to remove the tissues that transport sugars throughout the plant (the phloem), while leaving the water transporting tissue on the interior of the stem (the xylem). Girdling the branch produces two effects, the accumulation of sugars at the wound which stimulates the formation of roots, and while leaving the xylem intact means the branch will still have a constant supply of water.

Two cuts around the circumference of the branch were made. A knife was used since guava has a relatively tougher bark. Once the two cuts around the stem were made, a third cut was made straight down the stem, connecting the two circular cuts. The bark was then peeled off and the phloem (which was usually green) was completely removed. Every remaining green tissue was shaved off leaving the white xylem exposed.

The top of the wound was brushed with rooting hormone (Seradix no.3). A small handful of growing media was applied and excess water squeezed out since media had to be moist, but not dripping wet. Media was then wrapped around the girdled area and secured with a twine. The media was then tightly wrapped with plastic wrap and secured with two twist ties, at the top and bottom (Plate 1).

Plate1: An air layered West facing branch with top soil as a medium of rooting.

Step 4: Harvesting and transplanting

After mass of roots were seen through the plastic wrap, it was time to harvest the new plant. The branch was unwrapped, leaving the media in place (Plate 2). The branch was then cut where the original wound was made.

Plate 2: An unwrapped vermiculite, North East to South East oriented rooted branch.

2.5 Treatments: Factor 1, Main plot: media

There were four media treatments. The treatments were as follows: Vermiculite.

Ordinary top soil (sandy- loam)

Compost

Pre mixed media; pine sawdust, river sand and top soil (1:1:1)

Factor 2, Subplot: branch selection according to light intensity.

1. South West to South East facing branches.
2. North East to North West facing branches.

Treatment Combination: Table 1 shows the treatment combinations applied on each air layered branch. It also depicts the treatment combination codes.

Table 1: Description of treatment combinations

Treatment combination code	Treatment combination
1	Vermiculite + South East to North East oriented branch.
2	Top soil + South East to North East oriented branch.
3	Compost + South East to North East oriented branch.
4	Media mix + South East to North East oriented branch.
5	Vermiculite + North West to South West

	oriented branch.
6	Top soil + North West to South West oriented branch.
7	Compost + North West to South West oriented branch.
8	Media mix + North West to South West oriented branch.

Replications: there were 5 replications of each treatment to reduce error.

2.6 Data Collection: Data was collected once after rooting had occurred. Parameters recorded during data collection were volume of roots in terms of water displacement, mass of roots (fresh mass and dry mass), the length of roots, root number and rooting percentage.

Root Volume: Fully rooted marcots were sampled and roots, leaves and stems cut-off. A 100 ml beaker was filled with water and the volume of water recorded. The roots were then dipped into the beaker and the new volume of water was determined. Volume of roots was determined by the volume of water displaced by the roots.

Fresh and Dry Masses: Eight rooted air layered branches were sampled per tree at harvest. The plants were harvested; fresh mass or marketable yield was measured using a balance. This was done individually for roots, stems and leaves. To determine the dry masses these plants were oven dried at 70ºC for 48 h [17].

Average Root Length: Three individual roots were selected and their lengths determined and average root length was determined by adding the three root lengths and dividing the value by three. The ruler used was a 30 cm ruler.

Number of Roots: This was determined by counting the number of roots that had been formed after root initiation.

Rooting Percentage: This was determined by visually assessing branches that had rooted over the total number of branches air layered among the various treatments used and multiplied by 100.

2.7 Statistical Analysis: The data was subjected to Analysis of Variance (ANOVA) using MStat-C statistical package [18]. Where significant differences were detected means were separated using the Duncan's New Multiple Range (DNMRT) [19].

3.0 RESULTS.

3.1 Media Analysis Results: Results of media analysis for pH, available potassium and available phosphorus are shown in Table 2.

Table 2: Media analysis results

Sample name	pH (H_2O)	Available K mg/kg	Available *P* mg/kg
1.Vermiculite	7.3	141	5
2.Top soil	6.3	435	10
3. Compost	6.3	776	18
4. Pine bark	5.9	256	6
5. Sand	6.3	50	4

3.1 Root Volume: Root volume varied significantly ($P\leq 0.05$) among media treatments (Table 3). Vermiculite, wrapped branches rooted significantly higher ($P\leq0.05$) than the other treatments in terms of root volume (Table 4). This was followed by compost wrapped branches which were not significantly different from top soil treated branches (Table 4). Media mix wrapped branches rooted significantly lower ($P\leq0.05$) than the other media treatments.

North East to South East oriented branches resulted in significantly ($P\leq 0.05$) higher root volume when compared with South West to North West oriented branches across all the media (Table 3, 5).

3.2 Root Length: Root length varied significantly ($P \leq 0.05$) among treatments (Table 3). Vermiculite wrapped branches led to significantly higher root length in comparison with other media treatments used (Table 4). These were followed by top soil wrapped branches which however were not significantly ($P>0.05$) different in terms of root length as compared with media mix, top soil or compost wrapped branches. .

North East to South East oriented branches resulted in significantly ($P \leq 0.05$)) higher root length when compared with South West to North West oriented branches across the media treatments (Table 3, 5).

3.3 Root Fresh Mass: Root fresh mass varied significantly ($P \leq 0.05$) among media treatments with the highest fresh root mass being obtained from vermiculite (Table 3, 4). Compost wrapped branches ranked second followed by media mix and top soil wrapped branches that were not significantly ($P>0.05$) different from each other in terms of fresh root mass obtained (Table 4).

North East to South East oriented branches resulted in significantly ($P \leq 0.05$) higher root fresh mass when compared with South West to North West oriented branches across the media treatments (Table 3, 5).

3.4 Root Dry Mass: Vermiculite wrapped branches led to significantly ($P \leq 0.05$) higher dry root mass in comparison with all the other media treatments used (Table 3, 4). These were followed by compost wrapped branches which were not so significantly ($P>0.05$) different from vermiculite and top soil wrapped branches in terms of dry root mass but significantly different from media mix treated branches (Table 4).

North East to South East oriented branches resulted in significantly ($P \leq 0.05$) higher root dry mass when compared with South West to North West oriented branches across the media treatments (Table 3, 5).

3.5 Root Number: Number of roots of vermiculite treated branches was significantly (($P \leq 0.05$) higher when compared with other media treatments (Table 3, 4). Top soil wrapped branches ranked second but was not significantly different in terms of root number from compost and media mix air layered branches (Table 4).

North East to South East oriented branches resulted in significantly ($P \leq 0.05$) higher root number when compared with South West to North West oriented branches across media treatments (Table 3, 5).

3.6 Rooting Percentage: Rooting percentage was significantly ($P \leq 0.05$) higher in branches treated with vermiculite media as compared with the other media treatments (Table 5). Top soil and compost treated branches ranked second in terms of the percentage of rooted marcots and were not significantly ($p>0.05$) different from each other (Table 5). Percentage of rooted branches was lower in media mix wrapped branches and significantly ($P \leq 0.05$) lower when compared with other media treatments.

North East to South East oriented branches resulted in significantly ($P \leq 0.05$)) higher rooting percentage when compared with South West to North West oriented branches across the media treatments (Table 5).

Overall east oriented branches had higher values of growth parameters than west oriented branches (Table 6). On the other hand the medium vermiculite followed in decreasing order by compost, top soil and lastly media mix had higher values of growth parameters (Table 6).

4.0 DISCUSSION

The results indicate that vermiculite wrapped branches produced adventitious roots of a larger volume compared to media mix, top soil and compost. Root length of the adventitious roots formed were longer in vermiculite media treated branches. This superior performance of vermiculite was also evident in rooting percentage, root mass and root number of marcots formed. The second best performing media was compost in terms of root mass, root number and root volume and topsoil ranked second in terms of rooting percentage. Second best root length was observed in media mix wrapped branches even though it was the worst performing media in terms of root volume, root mass, rooting percentage and root number.

The success of vermiculite can be attributed to the physical and chemical properties of vermiculite that enhance adventitious root development during plant propagation. Parrotta [12] reported that vermiculite improves aeration and promotes greater penetration of roots thus the reason why vermiculite wrapped air layered branches were denser and longer compared to the other media used in this study. In their study Bita et al. [13] reported that there was a positive correlation between percentage proportion of vermiculite and total porosity. Mishra et al. [8] noted that for successful air layering of woody perennials such as litchi, media used should be loose and more porous to allow for denser and longer roots that can be able to withstand transition from air layering stage to further transplanting on natural soil for establishment of fruit trees.

Vermiculite's chemical property of possessing relatively high cation exchange capacity makes it to hold and make available to the growing plant ammonium, potassium, calcium and magnesium [12]. The presence of these elements creates a micro-climate that is favourable for adventitious root development making vermiculite more superior to top-soil, media mix and compost.

Media mix wrapped branches were less superior compared to other media treatments. This media was a mixture of sand, topsoil, and pine bark and the individual properties of the media mix components had an impact on the rooting of branches. Sand decreased the cation exchange capacity of the media mix and the top soil used was of a clay loam texture. One property of clay loam soil is that it is slowly impermeable thus the reason why the roots formed from this media were shorter and less dense. Also the reduction of the cation exchange capacity by sand made media mix to have compromised ability to hold and make available to the growing plant ammonium, potassium, calcium and magnesium which are cations essential for adventitious root formation in air layering [4; 7]. It should be noted though that the sand component in the media mix enhanced longer length root formation but not as long as vermiculite wrapped branches due to the presence of top soil which compromised impermeability of roots through the media mix.

South East to North East oriented branches had the greatest adventitious root development in terms of root length, volume, fresh and dry mass together with rooting percentage and root number as compared to South West to North West oriented branches. These results are in agreement with those of Mishra et al. [8] on litchi and Kong [20] who reported that branches should be oriented on South East to North East facing orientations where there is greater exposure to sunlight.

5.0 Conclusion

The results indicated a significant influence of propagation media on root formation on guava branches propagated by air layering. Vermiculite media gave rise to longer roots, greater root volume, mass and number together with higher rooting percentage.There was also a significant influence of branch orientation in relation to the sun on root development of guava branches propagated by air layering. North East to South East oriented branches produced adventitious roots of greater length, volume, mass, number and rooting percentage when compared with South West and North West oriented branches.Interaction between media and branch orientation was significant in all the parameters measured. A combination of wrapping branches with vermiculite and selecting branches on the South East to North East in relation to the position of the sun led to superior adventitious root formation in terms of root length, volume, mass and rooting percentage. An alternative second best combination was wrapping branches with compost and selecting branches on the South East and North East orientations.

6.0 **Recommendations**

It appears from this study that the most successful protocol of air layering is selection of branches from the South East to the North East orientations in relation to the sun since branches facing this direction have the greatest exposure to the sun and wrapping branches with vermiculite propagation medium. Further studies need to be carried out on other locally available media. This study needs to be repeated to compare results in different seasons.

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Table 3 Analysis of varience (ANOVA) for the effects of propagation media and branch orientation on growth parameters of air-layered guava(*Psidium guajava* L.) branches

A N A L Y S I S   O F   V A R I A N C E   T A B L E

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	4	3.190	0.798	1.1389	0.3586
2	Factor A	3	59.573	19.858	28.3593	0.0000
4	Factor B	1	14.762	14.762	21.0825	0.0001
6	AB	3	10.783	3.594	5.1331	0.0059
-7	Error	28	19.606	0.700		
	Total	39	107.914			

Coefficient of Variation: 21.12%

Grand Mean = 2.731   Grand Sum = 218.500   Total Count = 80

: Table 4: Effect of propagation media on growth parameters of air- layered guava (*Psidium guajava* L) branches.

Media	Root volume (ml)	Root fresh mass (g)	Root dry mass (g)	Root length (cm)	Rooting Percentage (%)	Root Number
Vermiculite	5.750a	8.340a	2.120a	15.69a	92.5b	25.9a
Top soil	3.140bc	3.540bc	1.108b	6.860c	50b	6.4b
Compost	4.380b	5.020b	1.565ab	6.580c	45b	5.4c
Media mix	2.580c	3.330bc	0.508c	6.730c	12c	1.7c

Mean values within the same column followed by the same letter are not significantly different from each other at $P \leq 0.05$. Mean separation by DNMRT.

Table 5: Effect of branch orientation on growth parameters of air-layered guava (*Psidium guaja*va L) branches.

Orientation	Root volume (ml)	Root fresh mass (g)	Root dry mass (g)	Root length (cm)	Rooting Percentage (%)	Root Number
South East to North East.	4.57b	5.430b	1.686ab	9.850b	61.250ab	11.7b
South West to North West	3.355bc	4.685bc	0.994bc	8.080bc	38.75b	8.0bc

Table 6: Effect of the interaction between propagation media and branch orientation on growth parameters of air-layered guava (*Psidium guajava*) branches.

Media	Orientation	Root volume (ml)	Root Fresh mass(g)	Root dry mass(g)	Root length (cm)	Rooting Percentage (%)	Root number
Vermiculite	East	6.900a	8.900a	2.646a	16.5a	100a	28a
	West	4.600b	7.780a	2.140ab	14.8a	85ab	23.8ab
Top soil	East	4.100bc	3.980bc	1.426cd	8.180b	60b	8.2b
	West	2.180d	3.100c	0.7900e	5.540bc	40bc	4.6bc
Compost	East	4.900b	5.580b	2.140ab	7.040b	60b	7.2b
	West	3.860bc	4.460bc	0.9900de	6.120bc	30bc	3.6bc
Media mix	East	2.380d	3.260c	0.4140e	7.680b	25bc	3.4bc
	West	2.780cd	3.100c	0.6020e	5.780	0	0

Mean values within the same column followed by the same letter are not significantly different from each other at P≤ 0.05. Mean separation by DNMRT.