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Effect of different substrates on the mass production of *vivo* plantlets of smooth cayenne cultivar of Pineapple (*Ananas comosus*) in the western highlands of Cameroon

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ABSTRACT

A study was conducted to investigate the potential of local waste materials as appropriate media for the propagation of smooth cayeenne pineapple (*Ananas comosus*). Nine propagation media replicated three times in a randomized block design included; soil, sawdust, compost, white wood chips, soil + sawdust, sawdust + compost, sawdust + white wood chips, compost + white wood chips and sawdust + compost + white wood chips. Defoliated stems obtained after harvest were splitted longitudinally and sown in substrates. Data on the number of suckers and microclimate show that the temperature and relative humidity within the propagators ranged between 22.30-30.70°C and 76-90%, respectively. Sucker initiation and development was faster and higher in the compost and compost-containing substrates. Compost produced the highest number of suckers (55) with 34.53%, 58.18% and 7.27% of suckers produced in the first, second and third month, respectively. Suckers produced during the first (6.33±0.33) and second months (17.00±3.78) were significantly higher ($P < 0.05$) than in the other substrates. White wood chips produced the lowest number of suckers (48) with 72.92% produced during the third month. However, the number of suckers produced three months after sowing for all the substrates showed no significant difference ($P > 0.05$).

Introduction

Pineapple (*Ananas comosus* var. *comosus*) is a perennial herbaceous monocot of family Bromeliaceae comprising 56 genera and 2,794 species (da Silva et al., 2013; Reinhardt et al., 2018). Pineapple originated in Brazil and cultivated species in the Amazon exhibit high genetic variability (Reinhardt et al., 2018). It is one of the leading tropical fruit commonly consumed as fresh and processed products such as pineapple juice due to its pleasant aroma, flavor and immense health benefits (da Silva et al., 2013; Hossain, 2016; Dennis and Okpeke, 2018; Difonzo et al., 2019). Other species in this family are used for ornamentals and fibre production (Reinhardt et al., 2018).

The most widespread variety is the smooth cayenne cultivar extensively cultivated in many tropical countries like Hawaii, Philippines, Australia, South Africa, Puerto Rico, Kenya, Mexico, Cuba and Formosa (Hossain, 2016). It is the third most important fruit in the tropics and subtropics after banana and citrus (Ma et al., 2012; Usman et al., 2013). In the past decade, world pineapple production has risen up to 27.4 million metric tons of which 5.44 million metric tons is being produced in Africa. Out of this, 1.28% is produced in Cameroon with the littoral as the leading region (FAOSTAT, 2017). Pineapple is cultivated worldwide in the tropics ranging from mild coastal climate for its edible terminal composite fruit consisting of coalesced berries. However, the expansion of pineapple cultivation for large-scale commercial production is limited by the lack of sufficient quality uniform planting materials (Ranawana and Eeswara, 2008; Agogbua and Osuji, 2011; Dennis and Okpeke, 2018).

Mass production of high quality, vigorous, uniform and clean planting material of pineapple in a relatively short period of time is possible through *in vitro* tissue cultural techniques but this procedure is cumbersome, expensive and the growth of seedlings is slow, requiring a long period of acclimatization in the greenhouses (Agogbua and Osuji, 2011; Yapo et al., 2011; Yapo et al., 2011; Mendoça et al., 2017). In Cameroon, pineapple has been propagated traditionally using vegetative parts such as slips, crowns, suckers arising from leaf axils and ratoons. This method is laborious with a low multiplication rate given the slow regeneration cycle of new suckers that is often accompanied by the prevalence of diseases and pests (Yapo et al., 2011).

In addition, the number of planting materials produced from a single mother plant is limited over a longer period of time (Ranawana and Eeswara, 2008). In Cameroon, pineapple-planting materials are suckers obtained from cultivated fields after harvesting the fruits. Suckers obtained from cultivated fields maybe infected, not uniform and the low multiplication rate may cause serious production gaps. The simple and cheaper split crown technique that does not require specialized skills, offers the opportunity to obtain multiple, clean and uniform plantlets to local farmers. The objective of this study was to determine the potential of local waste materials as appropriate substrates for vegetative propagation of pineapple out of the production.

Materials and methods

Study site

The experiment was conducted at the research and application farm (FAR) of the Faculty of Agronomy and Agricultural Sciences (FAAS) at the University of Dschang (UDs), West region of Cameroon. FAR is located at latitude 5.5° North and longitude 10.05° East at an altitude of 1410 m above sea level in agro-ecological zone III of Cameroon (Djeugap et al., 2015) (Fig. 1).

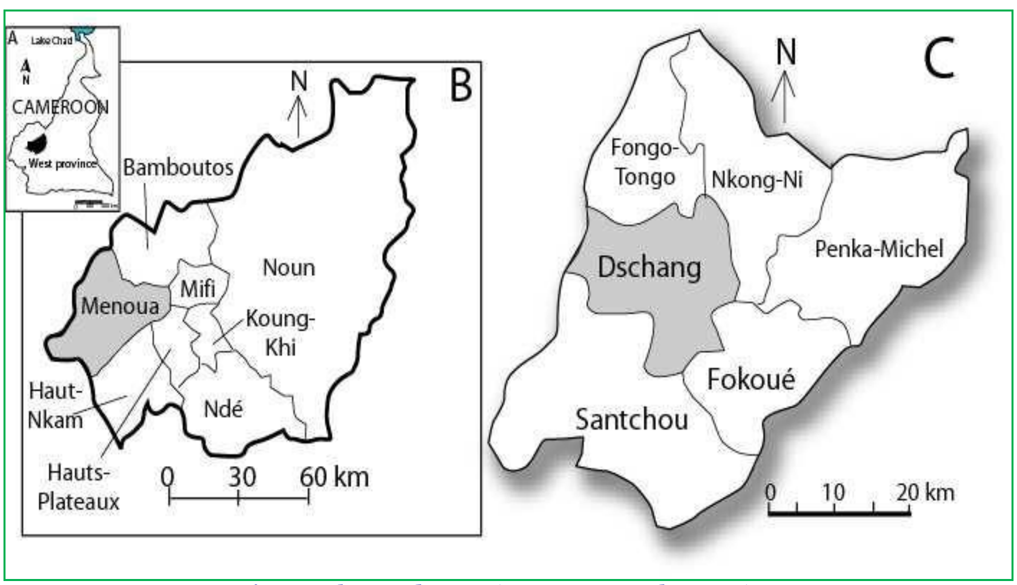

Fig. 1: The study site (Temgoua et al., 2012).

The vegetation of the exploited site is dominated by the following species: *Tithonia diversifolia, Mimosa pudica, Ageratum conyzoides, Ageratum haustonianum, Cyperus esculentus, Bidens pilosa* and *Pennisetum purpureum*.

The farm is located in the western highlands which is agro-ecological zone III characterized by a rainy season that runs from mid-March to mid-November and a dry season that runs from mid-November to mid-March. Mean annual rainfall varies between 1800 and 2000 mm and the mean annual temperature is around 20.03°C with maximum temperature ranging between 25 and 28°C in April and minimum temperature oscillating between 14 and 16 °C in December (Akongo, 2010).

Plant material

Plant material consisted of peeled pineapple stems of the smooth Cayenne variety bought from a pineapple producer at Nlohé in the Moungo Division, Littoral region. Pineapple stalks were obtained just after the harvest of 16 months old plants in the month January. Vigorous and disease-free stalks were selected and used in this experiment.

Preparation plant material for propagation

The leaves on the stalk of pineapple were carefully detached to expose the dormant axillary buds, which have the potential of developing into complete plants under favorable conditions. Leaflets, scales and roots were equally removed from the stalk. The bare stems obtained were then split longitudinally into two equal fragments. Splitting the stems ensured that none of the axillary buds were buried in the substrate, thus a possibility for all the buds to develop into complete plants.

Phytosanitary treatment

After splitting the stems, phytosanitary treatment using a nematicide (Counter 15 FC), a fungicide (Aliette 80 WG) and an insecticide (Dursban 4 E) was carried out to minimize diseases and insect attacks. A mixture was made in a 10 liters bucket containing 1 kg nematicide, 1 kg fungicide and 1 liter of insecticide. The stalk fragments were treated 24 hours before sowing by soaking them into this mixture and thereafter, dried under a shade. The mixture was also used to treat the sowing substrates before sowing.

Phytosanitary treatment was also carried out at the emergence of the first suckers. Thereafter, fungicide and insecticide application was done every fortnight.

Sowing media and experimental design

Nine sowing media (substrates) considered as different treatments were prepared using soil, sawdust, compost and wood chips at varying proportions were used in this experiment. The substrates were combined at various proportions to obtain varying density (0.10-0.90 g cm^{-3}), thus the water retention capacity (Table 1).

Table 1. Description of substrates used in pineapple macropropagation.

Substrate	Quantity experimental $unit^{-1}$	Density (g cm^{-3})
Soil	200 kg	0.90
Sawdust	23 kg (white wood)	0.15
Compost	90 kg mature city garbage	0.75
White wood chips	15 kg (white wood)	0.10
Soil + Sawdust	60 kg (4:1)	0.45
Sawdust + Compost	64.8 kg (1.4:1)	0.35
Sawdust + White wood chips	20 kg (1.5:1)	0.13
Compost + White wood chips	32.5 kg (2.3:1)	0.25
Sawdust + Compost + White wood chips	30 kg (6.3:4.2:1)	0.20

The experimental setup was a completely randomize block design where nine treatments (substrates) replicated three times. Macropropagation of pineapple was carried out in low tunnels of height 1.3 m (in front) and 1 m (behind). Three propagators of length 9 m and width 1 m were constructed using planks obtained from red wood. The planks had a rectangular base (5 cm long × 3 cm wide) and 24 pieces were used to construct the propagator. Planks were used as vertical supports while bamboos were used for support horizontally and at the sides.

The propagators were covered at the top and sides by cheap uv-stabilized polyethylene (thickness of 0.2 mm, light transmission of 92% at 90°). The propagators were separated from each other by a distance of 1 m and 1 m at the sides to allow free movements while working. Each propagator was considered as a block and was separated into 9 compartments of 1 m^2 (experimental units).

The quantity mentioned on table 1 of treated substrates was randomly attributed to the experimental units for each propagator. A shade of height 2 m was constructed over the entire experimental area to reduce brightness. Bamboos were used are vertical and horizontal supports while palm fronds were placed over the horizontal support to shade the propagators.

Sowing, irrigation and weeding

The peeled stem fragments weighing 2 kg were sown in each experimental unit giving a total of 54 kg for the three blocks. Heavy weight stems (>100g) were selected and used following the findings of Omotoso (2014). Stem fragments were sown at the depth of 3 cm, 10 cm between the lines and 5 cm along the line, with the sectioned part below and the intact part with buds above.

Experimental units were irrigated (11.25 mm) using a watering can at an interval of 4 days. Propagators were opened up in days when the internal temperature exceeded 28 °C and or when the internal relative humidity was above 90% for 1-4 hours (usually10:00-12:00 am). Manual weeding was conducted twice throughout the experiment on both the internal and external surroundings of the propagators.

Data collection and analysis

Daily climatic data (temperature and relative humidity) within and out of the propagators were measured throughout the study period at 7:00 am, 10:00 am, 12:00 noon and 18:00 pm. Agronomic data of number of suckers produced with respect to time and treatment (substrate) was obtained monthly through observation and counting. Data was subjected to descriptive statistics for mean values and analysis of variance (ANOVA) to test for the significance of effects of treatment on the parameters measured. Differences between treatment means were compared using Tukey test of SPSS version 21.

Results and discussion

Microclimate within the propagators

The highest temperature (30.70 °C) and lowest (22.30 °C) temperature inside the propagators were measured in the afternoon and early hours of the day, respectively. The highest temperatures were recorded at 12:00 and 18:00 pm daily. This was due to the sunshine that becomes intense and heat up the propagators in the afternoon periods (Fig. 2). Mean temperature inside the propagators throughout the study period was 27.2 °C (Fig. 2).

Relative humidity within the propagators varied throughout the day and throughout the experimental period. Generally, daily values of the relative humidity ranged between 76-92% with the lowest values recorded in the morning periods (7:00 am) when the sun has not heated the dew on the vegetation and the highest values recorded from 10:00 am due to the rising sun (Fig. 3).

Microclimatic conditions within the propagators play a vital role in the initiation and development of suckers from the sectioned stems within the various substrates. The temperatures recorded within the propagators are within the favorable diurnal temperature range (20-30°C) for optimal growth of pineapple (Hosain, 2016). Sucker initiation and growth from the sectioned stems relies on reserves stored in the stems. The use of these reserves relies on enzymatic-driven physiological processes of respiration and transpiration that are influenced by the climatic conditions of temperature and relative humidity. Various substrates further influenced the microclimate surrounding the stem fragments, thus the stem fragments respired differently and subsequently affected bud initiation and development.

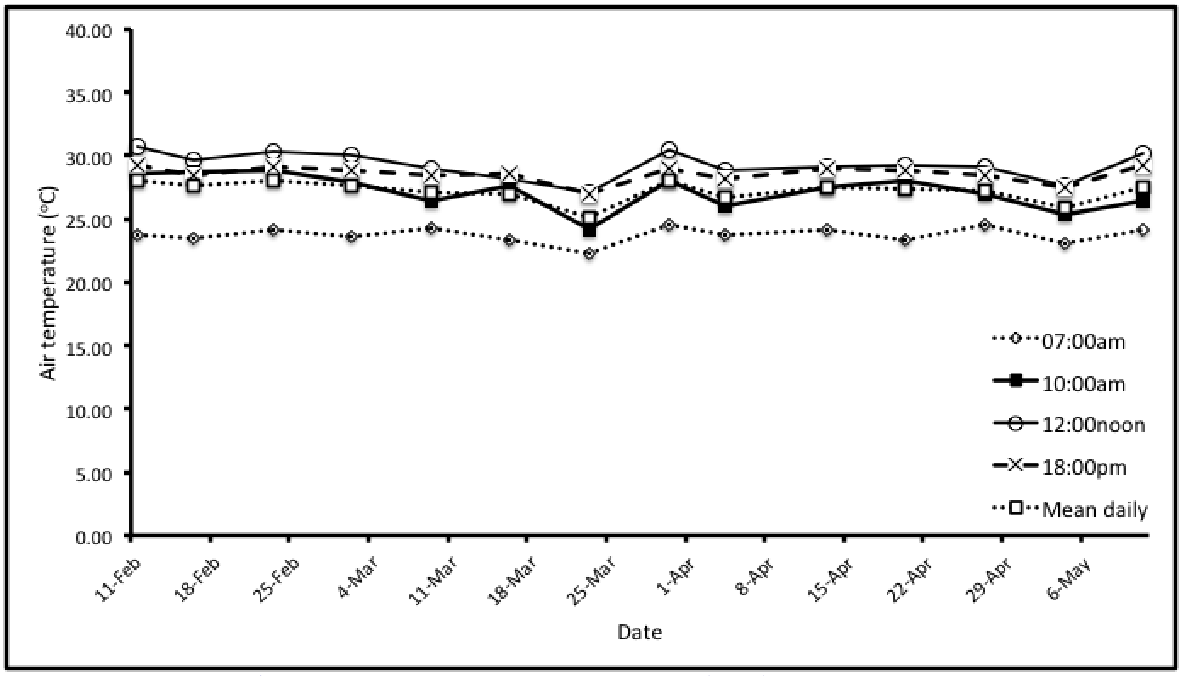

Fig. 2: Mean temperature variation within the propagators.

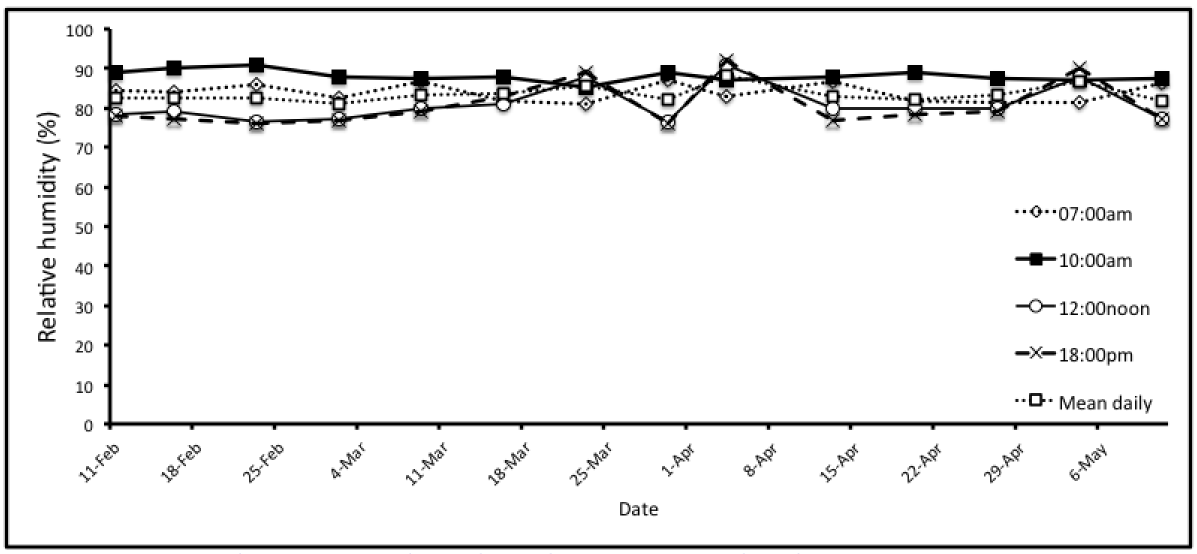

Fig. 3: Mean relative humidity variation within the propagators.

Agronomic data

Multiplication rate

Generally, the number of suckers initiated and developed on the stem fragments increased with time. The number of suckers initiated increased from the first month to the third month of this study (Fig. 4). Only experimental units or compartments containing compost produced suckers within one month of sowing with the substrate containing compost only producing the highest number of suckers (6.33±0.33). A similar trend was observed during the second month with compost producing (17.00±3.78) but this time around all the substrates produced suckers (Table 2). This resulted to significant differences ($p < 0.05$) in the mean number of suckers produced within substrates 60 days after planting (Table 2). The number of plantlets produced in the compost substrate reduced during the third month. This is probably due to the fact that suckers produced during the first to months were not separated from the mother stems to initiate the emergence of new suckers.

Also, the food reserves in the mother stem must have been exhausted by the previously initiated and growing suckers making it difficult for the initiation and development of new suckers. However, at the end of the third month, there was no significant difference ($p > 0.05$) amongst the substrates.

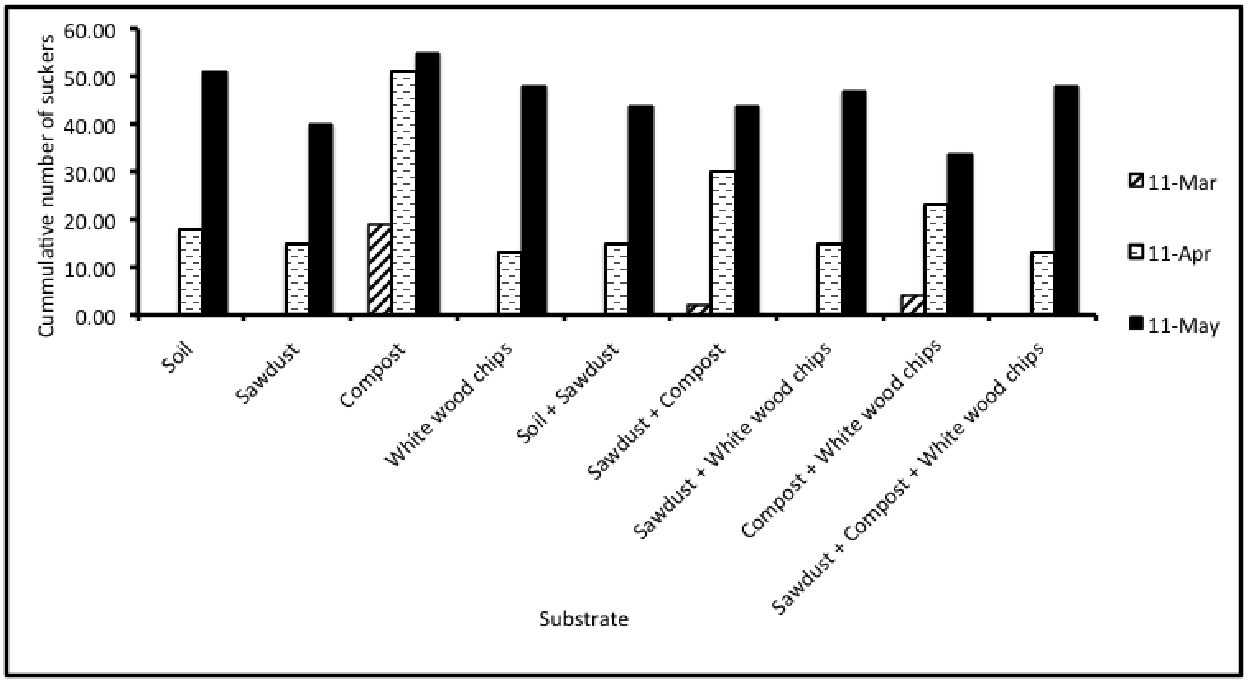

Fig. 4: Cumulative number of suckers produced throughout the study period.

Table 2. Temporal variation in the mean number of suckers produced per substrate.

Substrate	Number of suckers produced		
	11-March	11-April	11-May
Soil	$0.00±0.00^{c}$	$6.00±1.00^{b}$	$17.00±6.00^{a}$
Sawdust	$0.00±0.00^{c}$	$5.00±1.15^{b}$	$13.33±7.53^{a}$
Compost	$6.33±0.33^{a}$	$17.00±3.78^{a}$	$18.33±4.91^{a}$
White wood chips	$0.00±0.00^{c}$	$4.33±1.20^{b}$	$16.00±6.11^{a}$
Soil + Sawdust	$0.00±0.00^{c}$	$5.00±0.57^{b}$	$14.66±0.66^{a}$
Sawdust + Compost	$0.66±0.66^{bc}$	$10.00±0.57^{ab}$	$14.66±4.70^{a}$
Sawdust + White wood chips	$0.00±0.00^{c}$	$5.00±0.57^{b}$	$15.66±4.91^{a}$
Compost + White wood chips	$1.33±0.33^{b}$	$7.66±0.88^{b}$	$11.33±2.72^{a}$
Sawdust + Compost + White wood chips	$0.33±0.33b^{c}$	$8.66±1.45^{b}$	$17.33±2.60^{a}$

Means with the same letter within column are not significantly different at $p < 0.05$
±: standard error.

Previous studies showed that sucker initiation and development depends on variety, microclimatic conditions (growth media) and the developmental stage of the stems used (Pannetier and Lanaud, 1976; Ogunkule, 2014). Heterogeneity in the growth and development stage of the buds will influence their development (Ranawana and Eeswara, 2008). In this study, the variety used was the smooth cayenne cultivar and the stems were obtained at harvest and treated uniformly before sowing in the various substrates. The difference in the initiation and development of suckers observed during the first two months may have been due to the difference in the microclimatic climate around the stem fragments. Microclimatic conditions of temperature and relative humidity around the stem fragments were strongly influenced by the physical structure and texture of the substrates. Rapid initiation and development of suckers in the compost treatments was favored by the structure and the texture of the compost (density 0.73 g cm^{-3}); not too fine and not too coarse (Table 1). This is similar to the findings of Ogunkule (2014) who showed that the application of 0.002 T ha^{-1} fowl dung ensured faster and more plantlet generation producing 20 suckers within the shortest time (26.86 days), 19.75 and 17.50 suckers in 70 and 90 days after planting, respectively. The compost obtained may have provided superior structural stability compared to the other substrates during the first two months of the experiment.

In addition, the decomposition stage of the compost material may have strongly influenced the microclimatic conditions surrounding the stem fragments thus quickening the initiation and development of suckers. Temperature increments due to decomposition of the compost may have improved the microclimatic conditions better that favored sucker initiation and development faster than in the other substrates.

Conclusion

Results of this study demonstrate the potential of local wastes as suitable substrates for vegetative propagation pineapple within a period of three months. Compost produced from city waste proved to be the most appropriate substrate with 92.71% of the suckers produced within the first two months after sowing. In addition, other compost-containing substrates relatively produced suckers more than the other substrates. White wood chips produced the least number of suckers (48) with 72.92% produced in the third month. The results indicate that the structure, texture and decomposition stage of the city compost played an important role in the surrounding microclimate, which subsequently influenced the physiological processes involved in sucker initiation and development.

Other substrates constituting local was materials of sawdust, woodchips, soil and combinations of these materials produced suckers only during the second and third months following sowing. Sucker initiation and development in stem fragments sown in these substrates was very slow during the first month but increased during the third month. These initial results at the study site prove to be promising but we recommend further work to be carried out using the compost substrate at various decomposition stages with varying dimensions of the pineapple stem.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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