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The Effect of Float Seedling Growth Media Combination and Particle Size Distribution on Germination and Biometric Characteristics of Tobacco Seedlings (*Nicotina tabacum*)

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Abstract: An experiment was conducted for 20 weeks under float bed system in which nine tobacco seedling growing media mixed in different combinations based on particle size distribution of pine bark and coal rubble were studied. The objective of this study was to determine the effect of these different media combinations on the physical properties of the media blends; seed germination and biomass characteristics of tobacco seedlings in float tray system. The study was conducted at Kutsaga Research Station (17°52′ S; 31°02′ E, elev. >1500 m) near Harare in Zimbabwe. The experiment was designed as a Randomized Complete Block with three replicates. Study results show that media particle size distribution, combinations of pine bark and coal rubble media components had a comparatively significant effect on the total porosity of media blends while their influence on bulk density was largely insignificant. All media combinations in which the small to medium size pine bark particles (T_5 and T_8) contributed the bulk of media mass (90%) had the highest total porosities (70.2 and 70.1%) while the no-pine bark component treatment had the lowest (49.9%) total porosity (T_6). The media combination that generated a total porosity of nearly 50% (T_6) supported amplified initial germination counts. While a large body of research results elsewhere have indicated that the presence of sand or coal rubble components in media combinations tend to amplify spiral rooting counts of tobacco seedlings in float trays due to increased compaction associated with their high densities, our results have shown that it was the hydrology of the media combination that had a large influence on spiral rooting. The lowest initial tobacco germinations or increased seedling mortality places a reduced burden on the media nutrient resources thereby increasing the nutrient compensatory effect on growth of stems observed in T_3 float trays.

Key words: Media combinations, particle size distribution, tobacco seedlings

INTRODUCTION

For some time pine bark has been the most widely used growing media constituent for the production of tobacco seedlings in float bed and ebb-flood fertigation systems because of its suitable chemical and physical properties for propagating and growing plants (Ploeger, 1992). The search for suitable substitutes for pine bark media combinations with reduced content of pine bark has been prompted by concern over high costs, skewed availability coupled with increasing demand (Handreck and Black, 1993).

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Blending different seedling growing media components generates physical and chemical characteristics that are intermediate between properties of the components (Blom, 1993). For favourable physical characteristics to be present in the container media, total porosity should comprise over 50% of the media volume (Bilderback *et al*., 1999). The growing mix must be able to supply oxygen for proper root function, water and nutrients for shoot growth. The growing media mix must have adequate small pores to hold water for plant uptake and enough large pores to allow exchange of air in the medium to maintain critical oxygen concentration. A good media in the floating tray system should have the ability to wick water and retain it (Van Schoor *et al*., 1990).

The size of the particles and their distribution determine the texture of the media, which subsequently affects water retention and air porosity (Pasian, 2003). In pine bark medium small particles of less than 0.01 mm result in smaller pore diameter compared to that with large particles. These small pores increase the water holding capacity of the media and reduce air filled porosity (van Schoor *et al*., 1990). Particles between 0.01 and 0.08 mm retain most of the water applied. As particle size increases from 0.8 mm upwards the proportions of large non-capillary pores increases thus increasing the amount of space occupied by air after irrigation. Above 6 mm large non-capillary pores predominate (Puustjarvi and Robertson, 1975).

High water holding capacity and humid air spaces in the mix is important for germinating seedlings while good aeration and moderate water holding capacity are essential for long term planting (Percy, 1997). In general, Percy (1997) and Atland (1998) recommended that a good balance can be achieved with 2/3 to 3/4 coarse particle and the remainder occupied by medium particles.

In most pine bark based media 50% of water is available to plant while the other 50% is not available (van Schoor *et al*., 1990). Water that is unavailable to plant roots is bound to media forming a film over particles surface through a physical process called adhesion and cohesion referred to as Matric Potential (MP) (Atland, 1998).

Water holding porosity is the fraction of total pore space filled with water after free drainage and is expressed as a percentage. Pore size affects available water, drainage and the distribution of water in the container. Plant available water represents the water that is easily available to the plant roots.

When smaller particles of both pine bark and coal rubble are mixed with large particles, the pore diameter is reduced. Small particles of pine bark tend to lodge in the spaces left by larger particles thereby reducing the aeration porosity and increasing the water holding porosity (Pasian, 2003). When two media components have a large difference in the particle size the media usually shrink. This is due to small particles settling into large non-capillary pores located between large particles at the bottom of the container (Nash and Pokorny, 1990).

A loose porous mix with larger pore spaces would have a lower bulk density than a heavy compact growing mix. The only problem of a loose porous mix is that it does not provide adequate ballast for the plant and the plant may be top heavy. This can be solved by mixing heavy coarse aggregates with lightweight organic matter, thereby decreasing porosity and hydraulic conductivity and increasing bulk density (Holcomb, 1987). Sand can be added to a mix. Its dense particle nature and high bulk density provides ballast to keep top-heavy nursery crops upright. The physical characteristics of coal rubble media are relatively similar with those of the sand media. Consequently, coal rubble can be added to media combinations to perform the functions of sand particles in media combinations (Pasian, 2003).

Total porosity is defined as the volume proportion of space not filled with solids and it is directly proportional to bulk density. Porosity is simply the sum of air filled porosity and water holding capacity (Smith and Mullins, 1999). Total porosity is important in that it can be used for making calculations about the physical properties because it is the upper limit of the space in the medium. This space will be available for either water or air (Holcomb, 1987). Air filled porosity (AFP) is the proportion of the volume of medium that contains air after it has been saturated with water and allowed to drain. A good medium should drain excess water to reduce oxygen stress and avoid anoxic conditions. When small pine bark particles are used water is held in the bark and drainage is low. This can be amended by use of large particles, which improve the aeration and reduce the amount of water held in the medium (Van Schoor *et al*., 1990).

If the mix is very dense and there is too little air in the mix (less than 10%) then roots will not be properly aerated and the plant will suffer (Spiers, 2005). AFP of 10-15% is often water logged and algae that compete with plant for nutrients are common. AFP of 15-20% is ideal for nursery use. On the other hand AFP of more than 30% may be desirable for seed germination and ready to foster plant growth, especially in the floating tray system where water is always available (Van Schoor *et al*., 1990).

The effect of different media combinations on the growth and development patterns of tobacco seedlings in float tray systems has been widely studied. However, there has been inadequate research on the influence of container-media particle size distribution and combination on the biometric characteristics of tobacco seedlings, which are fundamental determinants of the field leaf yield and quality of tobacco. Accordingly, we report in this paper on an experiment led for 20 weeks in which nine tobacco seedling growing media mixed in different combinations based on particle size distribution of pine bark and coal rubble. The objective of this study was to determine the effect these different media combinations on the biomass characteristics of tobacco seedlings in float tray system. We reasoned that different particle size distributions in the combinations of pine bark/coal rubble based growing media presented variations in the seedling growing conditions in float trays, which caused the differences in the biometric characteristics of tobacco seedlings.

MATERIALS AND METHODS

The Field Study Site

The study was conducted at Kutsaga Research Station (17°52′ S; 31°02′ E, elev. >1500 m above sea level) near Harare International Airport in Zimbabwe. The soils at the research station are deeply weathered sandy loams derived from granite and classified as Udic Kandiustalf under the USDA system of soil classification (Nyamapfene, 1991). The area lies in Natural Region II receiving rainfall ranging from 800 to 1000 mm per annum (average 900 mm per annum) with a coefficient of variation of 19%. The mean annual temperature is 21°C with insignificant frost occurrence in the months of June and July. The rainfall occurs during a single rainy season extending from November to April. (Vincent and Thomas, 1960).

Media Preparation for the Trial

Media Components for the Treatments

The media mix consisted of composted pine bark commercially prepared in the Eastern Highlands of Zimbabwe and coal rubble collected from the tobacco barns after coal was burnt as a source of heat energy during tobacco leaf curing. The coal rubble media component was washed thoroughly with water to get rid of excess ash and allowed to drain and dry before crashing. The two media components were separately crushed using the builder’s rammer to pass a series of 0.5, 4 and 6 mm sieves after some extensive shaking. Consequently, three media particle size distributions were generated: small particles<0.5 mm, medium particles 0.5-4 mm and large particles 4-6 mm. Nine pine bark-coal rubble media combinations based on percentage particle size distribution and type in the treatment were generated after thorough mixing as shown in Table 1.

Table 1: Different media particle size mixes in the treatments

	Particle size distribution (%)		
Treatments	0.5-4 mm (70%)	<0.5 mm (20%)	4-6 mm (10%)
T_1 (control)	100% Coal medium particles		
T_2	Coal	Coal	Pine bark
T_3	Coal	Pine bark	Coal
T_4	Coal	Pine bark	Pine bark
T_5	Pine bark	Pine bark	Pine bark
T_6	Coal	Coal	Coal
T_7	Pine bark	Coal	Pine bark
T_8	Pine bark	Pine bark	Coal
T_9	Pine bark	Coal	Coal

Media Reaction

Samples of the seedling growth media were collected from each media mix for determination of pH and electrical conductivity (Gabriels and Verdonck, 1992) and subsequent adjustment of media reaction. The pH and EC analysis was done before sowing to allow for pH amendment using citric acid and lime. The pH value had to be in the range of 5.5 to 6.9.

Determination of Physical Properties of Seedling Growth Media Mix

Samples for each treatment were collected and oven dried for 24 h at 100°C. The samples were then analysed for bulk density, total porosity, aeration porosity and water holding capacity determination (De Boodt *et al*., 1973). The collected data was subjected to one-way Analysis of Variance (ANOVA) using GENSTAT software.

Field Experiment Set up

The field experiment was carried out in the floating trays under greenhouse conditions in order to determine the effect of nine different pine bark-coal rubble based growing media combinations and particle size distribution on germination and biometric characteristics of tobacco seedlings. The field experiment commenced with the construction of three floating beds of 6.07 m in length and 1.05 m in width each. The bricks were placed with a minimum of 2-brick coarse wall high. The entire construction was lined with 250 μ black plastic sheeting, which was at least 1.5 m wider and longer than the inside dimensions of the bed to allow for the plastic to be laid over the top of the wall to hold the plastic in place. The beds were then filled with water to a depth of 12-15 cm throughout. This effectively flattened the plastic against the sides of the pond and any wrinkles were pulled straight during this final exercise.

Different media combinations were pre-moistened for 18 h and left covered with a black plastic. This was done to allow media components to wick water before packing in float trays. Media was then mixed with water slowly on a flat working surface. A simple hand feeling test was done to ensure enough water had been added. A mix containing the correct amount of moisture holds its shape when squeezed into a ball for 2-3 sec before beginning to fall apart. Each tray was hand filled with the prepared media compositions by applying the standard methods. The trays were lifted to a height of about 20 cm above a flat surface and dropped gently. This slightly compacted the media in the trays. This was repeated 2-3 times with the trays being refilled after each drop. Under-packing result in media falling out through the holes at the bottom of the cells whilst over packing introduces problems with dibbling and spiral roots. The trays were then dibbled using a dibble board and sown with pelleted KRK26 tobacco seed cultivar. Each cell was then pressed at the centre to create depressions (dibbles) 1 cm deep. Trays were floated following a randomized order in each block noting the water level of each bed. Three 200 cell polystyrene trays were planted per treatment.

Treatments

The field trial was conducted during the 2004/2005 summer season. Nine composite media combinations were generated as shown in Table 1 to constitute nine treatments as follows: T_1-(control, farmer's present practice), T_2, T_3, T_4, T_5, T_6 T_7, T_8 and T_9.

The experiment was designed as Randomized Complete Block with three replicates, which were subjected to various combinations of pine bark-coal rubble matrices in order to determine their effect on growth and development of tobacco seedlings produced under floating tray system.

Management of the Field Experiment

The recommended basal fertilizer hydrofert with NPK 20:10:20 was used at rates of 150 mg L^{-1} of water split into three application rates of 25, 50 and 75 mg L^{-1} at 7, 21 and 35 days after sawing (DAS), respectively. Top dressing by ammonium nitrate to pond water was done at 42 days after sawing using a rate of 100 mg N L^{-1} of water. Tobacco seedlings were clipped by cutting off leaf tips to ensure uniformity and allow seedlings that lagged in growth and development to catch up with others. Clipping commenced at 5-7 cm height up to transplantable age.

Proper sanitation is critically important in preventing the introduction of disease into the tobacco transplant area. This was made effective by preventative measures. These included foot bathing and hand washing before entering the site and handling seedlings. Covering the beds every night when the temperatures were low and uncovering during the day provided favourable temperatures in the range of 25-30°. Thermometers were used to monitor temperature changes in the seedbed.

Data Collection

Measurements on dry cells and fall-outs were done 7 DAS. Germination and spiral roots counts commenced 14 DAS up to 35 DAS at weekly intervals. Spiral rooting is a condition when aerial roots develop as a result of a failure to go down due to compaction of the media or lack of sufficient oxygen in the media. Surviving and transplantable seedling were counted at 42 and 56 DAS when the experiment was terminated at 63 DAS. At termination of experiment a sample of seedlings was collected from the 50 centre cells of the tray. Stem length and diameter measurements were taken using a rule and veneer calipers, respectively. Stem diameter measurements were done just above the root crown. Analysis of Variance (ANOVA) was used to test the significance of treatments effects on the biometric characteristics of tobacco seedlings grown in the float bed using GENSTAT software.

RESULTS AND DISCUSSION

Results shown in Table 2 clearly indicate a comparatively distinct pattern ($f<0.01$) of media treatment effect on the total porosity of seedling growing media. However, media particle size distributions and combinations of pine bark and coal rubble did not have a significant effect on bulk density ($f>0.01$). Notably, all media combinations in which the small to medium size pine bark particles (T_5 and T_8) contributed the bulk of media mass (90%) had the highest total porosities (70.2 and 70.1%) while the no-pine bark component treatment had the lowest (49.9%) total porosity (T_6), which, incidentally recorded the highest bulk density of 1.32 g cm^{-3}. In separate studies on the physical properties of pine bark as a container media, Pasian (2003) and van Schoor *et al*. (1990) reported elevated water holding capacity in small diameter pores of pine bark media with less than 0.01 mm particle size compared with other soilless media components. A bulge in the water holding porosity of the media combinations with small to medium size particles of pine bark triggered a corresponding increase in the total porosity observed in this study. Present results show that the media combinations with the highest total porosities in T_5 and T_8 recorded correspondingly the highest

Table 2: Mean percentage of physical properties

Treatments	Particle size distribution (%)			Media physical properties			
	70% medium particles	20% small particles	10% large particles	Bulk density (g cm^{-3})	Total porosity (%)	Aeration porosity (%)	Water holding porosity (%)
T_1 (control)	100% Coal medium particles			1.18	55.0^{d}	33.7	21.3
T_2	C	C	P	1.07	57.7^{ce}	36.5	21.9
T_3	C	P	C	1.10	59.2^{eg}	33.2	26.9
T_4	C	P	P	1.06	57.8^{cef}	33.2	24.6
T_5	P	P	P	0.48	70.2^{a}	26.5	43.8
T_6	C	C	C	1.32	49.9^{b}	28.2	24.7
T_7	P	C	P	0.55	65.4^{b}	31.9	33.5
T_8	P	P	C	0.52	70.1^{a}	36.1	33.8
T_9	P	C	C	0.68	61.0^{gh}	29.7	31.3
Mean				0.88	60.7	32.1	29
F-test prob				0.0337	< 0.01	0.399	0.005
CV %				4.7	3.5	17.3	20.5
LSD				0.071	3.63156	9.6248	10.282
t (0.05,16)					= 2.12		

C: Coal rubble, P: Pine bark

Table 3: Mean germination counts (%) from day 14 to day 35 after sowing

Treatments	Particle size distribution (%)			Days after planting			
	70% medium particles	20% small particles	10% large particles	14	21	28	35
T_1 (Control)	100% Coal medium particles			18.5	32.3	45.3	43.2
T_2	C	C	P	38.5	40.5	46.2	49.2
T_3	C	P	C	11.3	43.5	51.8	53.6
T_4	C	P	P	33.3	27.0	32.5	57.8
T_5	P	P	P	12.5	45.7	45.0	56.8
T_6	C	C	C	47.5	62.2	56.2	65.8
T_7	P	C	P	11.8	36.3	39.7	51.0
T_8	P	P	C	19.3	30.0	47.0	51.8
T_9	P	C	C	24.7	34.2	44.7	48.1
Mean				24.2	39.1	45.4	53.0
F-test prob							0.002
LSD							9.871
t (0.05,16)					= 2.1		

water holding porosities of 43.8 and 33.8% and the least bulk densities of 0.48 and 0.52, respectively. It should be noted however, that such media combinations had subdued contents of aeration porosities, especially in the T_5 media where a paltry 26.5% aeration porosity was recorded.

Study results shown in Table 3 indicate that there was a comparatively discernible (f = 0.002) treatments effect on the germination counts of tobacco seedlings in float trays. T_6 float trays in which coal rubble constituted all three particle size ranges clocked the highest initial and final mean germination counts of 47.5 and 65.8%, respectively while T_3 and T_7 media combinations produced the least initial germination counts of a paltry 11.3 and 11.8% at 14 days after sowing before rapidly climbing to a modest 53.6 and 51.0%, respectively on the 35th day. A brief reference to results displayed in Table 2 clearly indicates that T_6 media combinations recorded a total porosity of 49.9%. This trend in the treatments effect on germination counts was not particularly surprising as it conformed to research results elsewhere. In a study on the effect of physical properties of composite container media materials on growth patterns of azalea Bilderback *et al*. (1999) concluded that a total

Table 4: Mean spiral rooting (%) from day 14 to day 28 after sowing

	Particle size distribution (%)			Days after planting		
Treatments	70% medium particles	20% small particles	10% large particles	14	21	28
T_1 (Control)	100% Coal medium particles			0.7	2.0	2.3
T_2	C	C	P	1.3	1.3	0.7
T_3	C	P	C	3.7	3.0	1.3
T_4	C	P	P	3.3	4.0	3.7
T_5	P	P	P	3.0	1.7	3.0
T_6	C	C	C	0.0	2.6	0.0
T_7	P	C	P	1.7	4.0	1.3
T_8	P	P	C	1.3	6.0	5.3
T_9	P	C	C	3.0^b	4.0	1.7
Mean				2.00	3.2	2.2
F-test prob						0.004
LSD						1.293
t (0.05)						= 2.12

porosity constituting 50% of media volume generates favourable physical characteristics which enhanced germination of tobacco seeds observed in T_6 trays in this study. Results on the effect of various combinations of pine bark and coal rubble media components on germination counts were less convincing and did not show a definite trend.

None the less, a comparison of results on germination counts in the control trays (T_1, 100% medium size particles of coal rubble) and in T_6 trays (70% medium, 20% small and 10% large particles all from coal rubble) distinctly show that varying the distribution of particle sizes in the media amplifies the germination counts of tobacco seeds by about 7.8%. When smaller particles of growing media are mixed with large particles, the pore diameter is reduced (Pasian, 2003). Small media particles tend to lodge in the spaces left by larger particles reducing the aeration porosity by 5.2% and increasing the water holding porosity by 3.4% in the T_6 trays when compared with the same parameters in the control trays.

Data presented in Table 4 show spiral rooting percentages of tobacco seedlings between 14 and 28 days after sowing. Various combinations of coal rubble and pine bark components coupled with different particle size distributions had a significant effect on the spiral rooting of tobacco seedlings ($F<0.01$). Generally, spiral rooting significantly dwindled towards pulling stage ($F<0.05$) probably because spiral rooting is a compensatory mechanism that the seed adopts when the radicle fails to penetrate the media. With time, however, the spiralled roots successfully penetrated the relatively compacted media following the phenomenon of negative geotropism, which effectively nullified spiral root occurrence. None the less, the highest spiral rooting counts were recorded in the media cocktail with 20% pine bark particles <0.5 mm, 70% pine bark particles 0.5-4 mm and 10% coal rubble particles 4-6 mm diameter (T_8). In T_8 trays, initial spiral rooting clocked a relatively low count of 1.3% on 14 days after sowing before rapidly climbing to 6.0 and 5.3% in the third and fourth week after sowing. Tobacco seedlings in T_6 and T_3 float trays had the lowest spiral rooting percentages of 0.0 and 0.7% recorded on the 28th day after sowing.

In a study conducted at Pietermaritzburg, South Africa, on the preparation and utilization of pine bark as a medium for plant growth van Schoor *et al*. (1990) reported reduced pore diameter in pine bark media with particles <4 mm diameter which amplified water holding capacity with a net reduction in air filled porosity. This, inevitably, has the effect of generating anoxic (anaerobic) conditions in the float trays conducive for the development of spiral rooting in tobacco seedlings observed in this study.

Table 5: Mean growth parameters at pulling stage

	Particle size distribution (%)			Biometric characteristics		
Treatment	70% medium particles	20% small particles	10% large particles	No. of leaves	Stem length (cm)	Stem diameter (cm)
T_1 (Control)	100% Coal medium particles			6.269	11.6	0.3478
T_2	C	C	P	6.596	19.2	0.3264
T_3	C	P	C	6.363	20.2	0.3812
T_4	C	P	P	6.342	14.9	0.2894
T_5	P	P	P	5.850	15.5	0.3082
T_6	C	C	C	6.473	17.1	0.3450
T_7	P	C	P	5.677	15.9	0.2949
T_8	P	P	C	6.980	14.3	0.2585
T_9	P	C	C	5.808	13.5	0.2948
Mean				6.155	15.8	0.3163
F-test prob				0.226	0.003	0.146
LSD				0.792	1.308	0.083
CV%				8.2	5.6	16.5
t (0.05)				= 2.12		

The fact that media treatments that produced the highest bulk densities (1.32) in T_6 trays where conditions for media compaction that should have caused increased spiral rooting did not generate elevated spiral rooting convincingly show that media hydrology was the largest determinant of spiral rooting in our study.

It has been reported in numerous studies on container media that the sand component in growing media combinations tends to increase both the bulk density and tendency for compaction of the media thereby increasing opportunity for spiral rooting. Pasian (2003) reported that the physical characteristics of coal rubble media are relatively similar with those of the sand media. Consequently, coal rubble can be added to media combinations to perform the functions of sand particles in media combinations. In this study, however, coal rubble-based media cocktails in T_6 and T_2 had the least spiral rooting percentages. We reasoned that it is the media hydrology that significantly determines spiral rooting while the bulk density and tendency for compaction has a subdued influence on it.

Results shown in Table 5 indicate that there was a clear trend in the media treatment effect on the stem lengths ($F<0.01$) while data on number of leaves and stem diameters of tobacco seedlings at pulling stage did not show a definite pattern ($F>0.01$). T_3 media combination in which 70, 20 and 10% of the media blend were contributed by medium coal rubble, small pine bark and large coal rubble particle sizes, respectively, propagated the tallest tobacco seedling stems in the float trays. T_3 seedlings were 8.6 cm taller than those in the control plots. Of particular interest was the fact that initial germination counts at 14 days after sowing (Table 3) in the T_3 float trays were the lowest amongst all the treatments (11.3%). Indeed, this coincidence of the tallest stems and lowest germination counts in T_3 float trays was not accidental. We reasoned that the subdued initial germination counts in these trays placed a lower burden on the growing media nutrient resources thereby encouraging a vigorous root development and growth that triggered a bulge in the above-media-surface biomass accumulation. The tallest stems observed in T_3 were attributed to the nutrient compensatory effect on fewer seedlings that emerged 14 days after sowing. This was because when fertilizer was added to the float pond calculations were made for 100% germination and survival.

In conclusion, study results show that media particle size distribution, combinations of pine bark and coal rubble media components had a comparatively significant effect on the total porosity of media blends while their influence on bulk density was largely insignificant. One particularly interesting finding in this study was the fact that all media combinations in which the small to medium size pine bark particles (T_5 and T_8) contributed the bulk of media mass (90%) had the highest total porosities (70.2 and 70.1%) while the no-pine bark component treatment had the lowest (49.9%) total porosity

(T_6). The media combination that generated a total porosity of nearly 50% (T_6) supported amplified germination counts, a finding in this study that confirmed observations by Bilderback *et al*. (1999) on growth patterns of azalea. While a large body of research results elsewhere have indicated that the presence of sand or coal rubble components in media combinations tend to amplify spiral rooting counts of tobacco seedlings in float trays due to increased compaction associated with their high densities, our results have shown that it was the hydrology of the media combination that had a large influence on spiral rooting. The lowest initial tobacco germinations or increased seedling mortality places a reduced burden on the media nutrient resources thereby increasing the nutrient compensatory effect on growth of stems observed in T_3 float trays. However, considering that tobacco production is a business venture that must create profits for sustainability, our study could have been more encompassing if it had included the economic comparison of the nine media combinations.

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