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Chemical root-pruning of *Moringa oleifera* for improved seedling growth

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ABSTRACT

Low survival rates in greenhouse raised *Moringa oleifera* seedlings after transplanting had been ascribed to rapid growth rates and poorly formed and etiolated stems. The objective of the study was to determine the effect of indole acetic acid (IAA) on above-ground vegetative growth of *M. oleifera* seedling when used as root pruning agent. Hardened-off uniform one-month-old *M. oleifera* seedlings were transplanted in 20-cm-diameter plastic pots containing pasteurised loam soil and Hygromix at 3:1 (*v*/*v*) ratio. A week after transplanting, IAA concentrations of 0.0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6 and 51.2 mg mL^{-1} distilled water with G49 wetting agent and sticker were applied weekly to the respective plants. The effects of IAA concentrations were highly significant ($P \le .01$) on plant height, leaf number, dry root mass and significant ($P \le .05$) on stem diameter, which were further subjected to the lines of the best fit. Leaf number, plant height and stem diameter over increasing IAA concentrations exhibited positive quadratic relations, with the relationships being explained by 99, 98 and 98%, respectively. However, dry root mass and increasing concentrations of IAA exhibited negative quadratic relations, with the relationship being explained by 98%. At low concentrations, IAA increased plant height, number of leaves and stem diameter, whereas, at high concentration, the material reduced plant variables. However, all concentrations used reduced dry root mass. In conclusion, IAA was able to reduce root growth and promote shoot growth, with optimum for *M. oleifera* seedling growth established at a concentration level of 1.98 mg mL^{-1}.

1. Introduction

Moringa oleifera, indigenous to India, is a multi-use plant for improving the well-being and nourishment in marginal societies and had been the most favourable candidate from which precise nutraceutical bioactive products might be industrialised (Hassan and Ibrahim, 2013; Udechukwu et al., 2018). Thirteen *Moringa* species within the *Moringaceae* family have been documented, with *M. oleifera* being the most commonly well-known and used species (Amaglo et al., 2010; Paliwal et al., 2011). Subsequently, due to its numerous benefits, the plant had been given names such as miracle tree, horseradish tree, mother's best friend, and drumstick tree (Lekgau, 2011; Makita, 2014).

However, survival challenges of seedlings had been encountered due to its high growth rates (Agyepong, 2009). Low survival rates in greenhouse grown *M. oleifera* seedlings after transplanting had been ascribed to rapid growth rates and poorly formed etiolated stems (Goss, 2007; Pant, 2016). Consequently, the seedling stems are occasionally not strong enough to carry the foliage, resulting in low survival after transplanting (Antwi-Boasiako and Enninful, 2008). Also, during establishment, seedlings have shown symptoms of stunted growth and yellowing of leaves, resulting in death or reduced growth (Pahla et al., 2013). The low survival rates discourage the establishment of *M. oleifera* as an economic crop by smallholder farmers and marginal communities in South Africa (DAFF, 2012).

Several studies on *M. oleifera* focused mainly on high nutritive values and vast medicinal properties of the plant than on its agronomy as a cultigen (Gopalakrishman et al., 2016; Saini et al., 2016). Even though some *M. oleifera* production studies in South Africa are underway (Mabapa et al., 2017; Mashela, 2017), literature on its agronomic aspects is based mainly on Indian studies where the crop originates (Otun, 2015) and there is a little documentation on the strategies for establishment of strong seedlings (Goss, 2012).

Root pruning has been used extensively as a cultivation technique in the regulation of above and below-ground plant sections to influence the vegetative growth (Asin et al., 2007; Yang et al., 2009). A lot of advantages have been reported on root pruning which includes increased water-use efficiency (Ma et al., 2009; Wang et al., 2014), improved fruit quality (Yang et al., 2011), increased nodule growth (Cazenave et al., 2014) and increased leaf nutrients and photosynthesis (Jing et al., 2017). There are basically two approaches that have been researched and used in plant root-pruning, *viz*; physical and chemical root pruning. Physical root pruning is labour intensive and exposes the plant to a range of soil-borne pathogens (Ma et al., 2009), whereas chemical

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root pruning when heavy metals are used on food crops has the potential of exposing the consumers to health risks associated with heavy metals and these can also be leached into ground water causing environmental pollution (Kaub et al., 1998).

Literature is full on reports of plant growth regulators (PGRs) used to influence plant growth (Kozlova et al., 2018; Mohite, 2013; Sadak et al., 2013). Indole acetic acid (IAA) is the most abundant naturally occurring PGR and most studied (Abbas, 2013). Exogenous IAA applications have been reported to improve germination, growth, fruit setting, fresh vegetable and seed yield quantity and quality (Abbas, 2013; Dilfuza, 2011; Kozlova et al., 2018). Besides directly influencing plant growth, IAA has been observed to alleviate plants from several adverse biotic (Gravel et al., 2007; Sharaf and Farrag, 2004) and abiotic stresses (Afzal et al., 2005; Ma et al., 2009; Wang et al., 2014). Despite all the vast array of potential uses of IAA reported, there is limited information on the use of the plant hormone in root pruning and none on its potential for root pruning on *M. oleifera.* The objective of this study was therefore to determine the effect of IAA on above-ground vegetative growth of *M. oleifera* seedlings and establish optimal IAA concentration for use as root pruning agent.

2. Materials and methods

2.1. Plant growth conditions and preparation of materials

The study was conducted under greenhouse conditions at University of Limpopo, South Africa (23°53′10″S, 29°44′15″E). The trials were conducted between summer and autumn (January–April) 2016. *Moringa oleifera* cv. 'PKM1' seeds and IAA were obtained from the Roodeplaat Agricultural Research Council — Vegetable and Ornamental Plants (ARC — VOP). Twenty-cm-diameter plastic pots were each filled with 7 L steam-pasteurised sandy loam and Hygromix growing mixture at 3:1 (*v*/*v*). Pots were placed on greenhouse benches at inter- and intra-row spacing of 0.3 m. Seeds were sown into the seedling trays filled with Hygromix growing mixture. Hardened-off uniform one-month-old *M. oleifera* seedlings were transplanted into pots.

2.2. Experimental design, treatments and cultural practices

Ten concentrations of IAA, namely, 0.0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6 and 51.2 mg mL^{-1} dissolved distilled water, were arranged in a randomised complete block design, with five replications. Plants were fertilised a day after transplanting with 2.5 g of 2:3:2 (22) fertiliser mixture per plant to provide a total of 155 mg N, 105 mg P, and 130 mg K per ml water and 2 g of 2:1:2 (43) Multifeed (Nulandies, Johannesburg, South Africa) to provide 0.47 mg N, 0.43 mg K, 0.43 mg P, 1.21 mg Mg, 1 mg Fe, 0.10 mg Cu, 0.47 mg Zn, 1.34 mg B, 4.02 mg Mn and 0.09 mg Mo per ml (Tseke et al., 2013). Every other day each plant was irrigated with 250 mL tap water. A week after transplanting, IAA with G49 wetting sticker (1 ml L^{-1}) were applied weekly to the respective seedlings through foliar application.

2.3. Data collection

At 83 days after initiating the treatments, plant height was measured from the surface of the soil to the tip of the flag leaf and number of leaves per plant were counted. Average chlorophyll content of three matured leaves per plant were measured using chlorophyll meter (Minolta Spad-502). Shoots were severed from roots and stem diameter was measured using Vernier calliper at 5 cm from the cut end of stem. Root systems were removed from pots, immersed in water to remove soil particles and blotted dry with paper towel. Shoots and roots were oven-dried at 70 °C for 72 h and then weighed to obtain dry mass.

2.4. Data analysis

Data on plant growth were subjected to analysis of variance using SAS software (SAS Institute, 2010). Data on number of leaves per plant were transformed through $\log_{10}(x + 1)$ to homogenise the variance (Gomez and Gomez, 1984) before analysis, but untransformed means were reported. The degrees of freedom and their associated sum of squares were partitioned to provide the total treatment variation (TTV) for the different sources of variation. Mean separation was achieved through the Waller–Duncan multiple range test. Significant plant variables were subjected to lines of the best fit, using the relation $x = -b_1/2b_2$ concentrations from quadratic relation $Y = b_2x^2 + b_1x + c$, with quadratic equations generated to compute optimum concentration values. The IAA concentrations were $\text{Log}_2(10x)$ transformed before plotting the graphs to create equidistance between the concentration levels. Untransformed optimum concentrations were presented. Unless stated otherwise, treatments discussed were significant at 5% probability level.

3. Results and discussion

According to Liu et al. (2016), IAA concentrations had no significant effect on seedling root and shoot growth of Chinese cork oak seedlings. Noda et al. (2000) reported similar responses on roots of citrus root stocks at high concentrations of IAA. These observations contradict the current study where IAA concentrations were highly significant ($P \le .01$) on leaf number, plant height and dry root mass, and were significant ($P \le .05$) on stem diameter (Table 1) but had no effect on chlorophyll content and dry shoot mass (Table 2). Treatments contributed 73, 78, 54 and 81% in TTV on leaf number, plant height, stem diameter and dry root mass, respectively (Table 1). Observations in the current study supported findings made by Goudjal et al. (2013) and Zhao and Hasenstein (2009) who reported significant effects of IAA on growth of tomato plants and flax, respectively.

At lower concentrations, IAA stimulated all plant growth variables whereas at higher concentrations it reduced them, except for dry root mass (DRM) which was reduced at all tested concentrations (Table 3). Relative to untreated control, number of leaves, plant height and stem diameter were stimulated by concentrations below 1.6, 3.2 and 25.6 mg mL^{-1}, respectively (Table 3). Of all the plant variables

Table 1
Partitioning sum of squares for the effect of indole acetic acid on leaf number, plant height, stem diameter and dry root mass of *Moringa oleifera*.

		Leaf number		Plant height		Stem diameter		Dry root mass	
Source	DF	SS	%	SS	%	SS	%	SS	%
Replication	4	0.05	14	1927.63	22	124.45	45	94.70	13
Treatment	9	0.29	78***	6386.62	73***	150.06	54**	568.79	81***
Error	36	0.03	8	418.26	5	2.33	0.8	40.35	6
Total	49	0.37	100	8732.51	100	276.84	100	703.84	100

** Significant at $P \le .05$.
*** Highly significant at $P \le .01$.

Table 2
Source of variation as affecting plant dry shoot mass and chlorophyll content *Moringa oleifera*.

		Dry shoot mass		Chlorophyll content	
Source	DF	MS	%	MS	%
Rep	4	223.312	72	46.6567	40
Treatment	9	56.609	18ns	24.5263	21ns
Error	36	31.359	10	44.9833	39
Total	49	311.280	100	116.1663	100

ns, Not significant at $P \le .05$.

Table 3
Influence of indole acetic acid on leaf number (NOL), plant height (PHT), stem diameter (SDM) and dry root mass (DRM) of *Moringa oleifera*.

Treatment	NOL [x]	Impact (%) [z]	PHT (cm) [x]	Impact (%) [z]	SDM (mm) [x]	Impact (%) [z]	DRM (g) [x]	Impact (%) [z]
0	16.40[c]	–	93.64[cd]	–	12.63[a]	–	15.98[b]	–
0.2	16.80[c]	2	105.00[ef]	12	14.28[ab]	13	11.86[a]	−26
0.4	17.00[c]	4	106.40[f]	14	14.80[b]	17	12.62[ab]	−21
0.8	16.90[c]	3	106.10[f]	13	14.80[b]	17	12.96[ab]	−19
1.6	16.80[c]	2	103.10[ef]	10	14.93[b]	18	12.96[ab]	−19
3.2	15.80[bc]	−4	97.70[de]	4	15.03[b]	19	13.09[ab]	−18
6.4	14.60[abc]	−11	92.70[cd]	−1	14.71[b]	16	12.78[ab]	−20
12.8	15.24[bc]	−15	88.90[bc]	−5	14.24[ab]	13	12.14[ab]	−24
25.6	13.00[ab]	−20	83.90[ab]	−10	13.25[ab]	5	11.86[a]	−26
51.2	11.40[a]	−30	78.90[a]	−16	12.39[a]	−2	10.80[a]	−32

[x] Column means followed by the same letter were not different ($P \le .05$) according to Waller—Duncan multiple range test.
[z] Impact (%) = [(treatment/control) − 1] × 100.

measured, stem diameter had the least unpropitious concentration levels of IAA for plant growth, whereas dry root mass all used concentrations suppressed the variable (Table 3).

Malik and Sindhu (2011) also reported variations in response of plant growth to different IAA concentrations. Goudjal et al. (2013), Malik and Sindhu (2011) observed a promotion of root growth of tomato and chick pea, respectively, at high concentrations with inhibitions at lower concentrations, contradicting with the current study. The contradictions could be explained by report that different plants and plant organs respond differently to different chemicals (Dube, 2016). These references in responses would be due to the differences in the anatomy and physiology of different plants that help them to deal with environmental conditions.

In the current study, relative impact values of plant growth variables when plotted against increasing IAA concentrations generated density-dependent growth (DDG) patterns (Figs. 1—4). The DDG

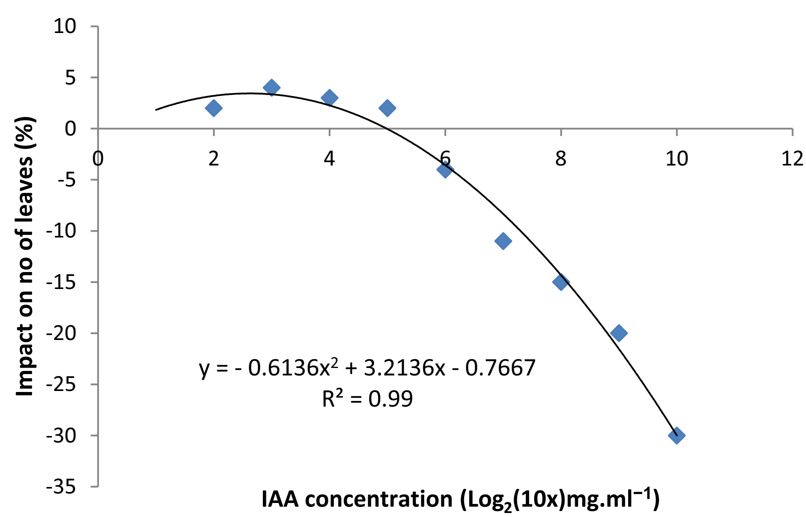

Fig. 1. Response of leaf number of *Moringa oleifera* seedlings to concentrations of indole acetic acid.

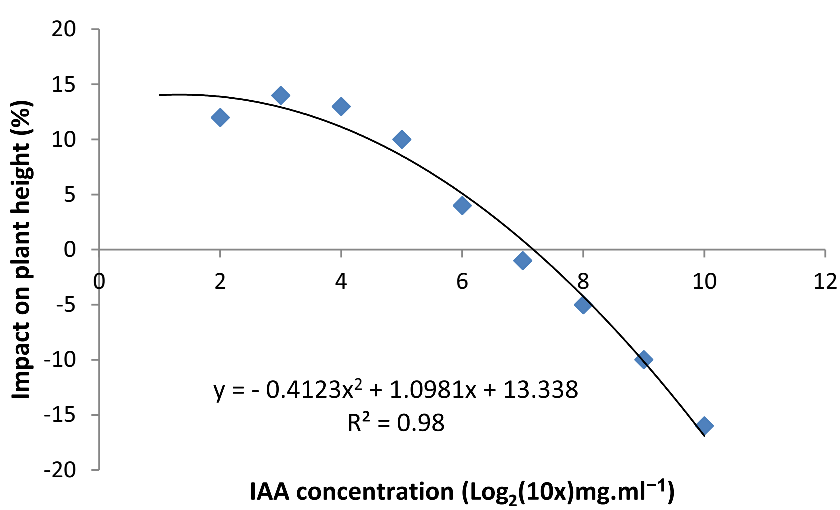

Fig. 2. Response of plant height of *Moringa oleifera* seedlings to concentrations of indole acetic acid.

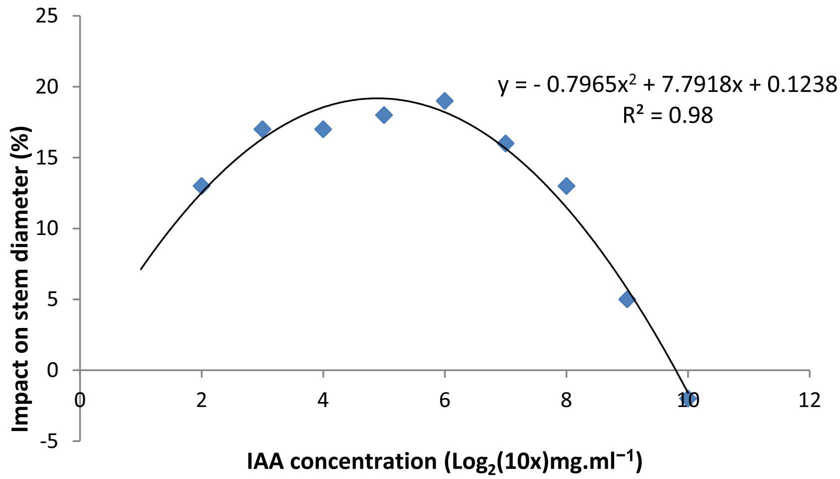

Fig. 3. Response of stem diameter of *Moringa oleifera* seedlings to concentrations of indole acetic acid.

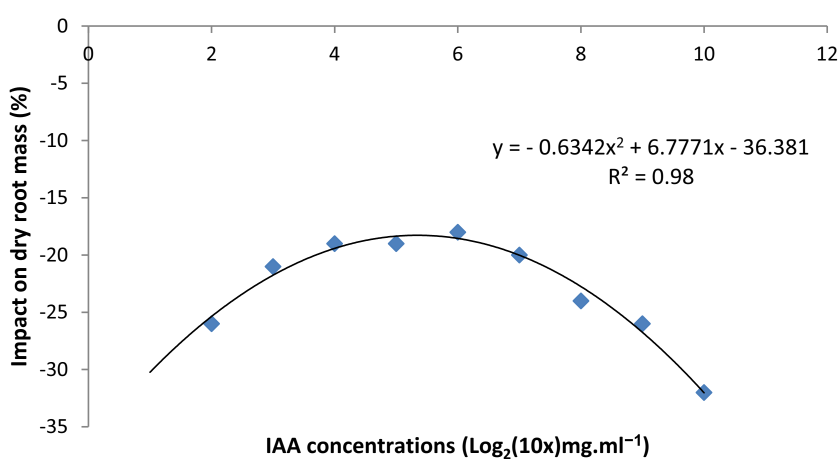

Fig. 4. Response of dry root mass of *Moringa oleifera* seedlings to concentrations of indole acetic acid.

patterns had stimulation and inhibition effect at low and high IAA concentrations, respectively. Number of leaves, plant height, and stem diameter over increasing IAA concentrations exhibited positive quadratic relations, with relationship being explained by 98, 99 and 98%, respectively (Figs. 1 — 3, Table 4). However, IAA concentrations exhibited negative quadratic relations with the model being explained by 98% on dry root mass (Fig. 4). The optimal for IAA concentrations on number of leaves, plant height, stem diameter and dry root mass were 0.61, 0.25, 2.99 and 4.05 mg mL^{-1} respectively, with mean optimum value of 1.98 mg mL^{-1} water (Table 4). In most trees, root flushes alternate with shoot flushes (Hartmann and Opitz, 1977), which could explain the improved shoot growth

Table 4
Quadratic relationship, coefficient of determination and computed optimum response concentration of indole acetic acid for leaf number (NOL), plant height (PHT), stem diameter (SDM) and dry root mass (DRM) of *Moringa oleifera*.

Organs	Quadratic relation	R^2	x ($mg.mL^{-1}$)
NOL	$Y = -0.6136 \times {}^2 + 3.2136x - 0.7667$	0.99	0.61
PHT	$Y = -0.4123 \times {}^2 + 1.0981x + 13.338$	0.98	0.25
SDM	$Y = -0.7965 \times {}^2 + 7.7918x + 0.1238$	0.98	2.99
DRM	$Y = -0.6342 \times {}^2 + 6.7771x - 36.381$	0.98	4.05
Mean optimum value			1.98

$x = -b_1 /2b_2$, where $Y = b_2x^2 + b_1x + c$.

at the expense of root growth in *M. oleifera* seedlings in the current study. Slightly similar findings were reported by Arnold and Struve (1989), which indicated that pruning roots reduced dry root mass of *Flaxinus pennsylvanica* seedlings and shoot extension in *Malus domestica* seedlings. On the contrary, Bath et al. (2008) indicated that two root pruning treatments significantly increased dry shoot mass of white cabbage. Root pruning had less adverse effects on growth of apple than on green ash seedlings after transplanting (Arnold and Young, 1991). Beyeler et al. (1999) highlighted that concentration range in which IAA had positive effect on root growth of corn and wheat were very low and higher concentration had negative effect on roots. Other studies observed that root pruning treatments significantly affected the height and diameter at breast height (DBH) growth of poplar trees. Moreover, compared to control, the increment of DBH was significantly increased after moderate root pruning (DesRocher and Tremblay, 2009).

However, the current results contradicted those by Guan et al. (1997), who indicated that lower IAA concentrations increased root growth of apple tree (*Malus domestica* Borkh). Malik and Sindhu (2011) reported the reversal of the concentration roles from the current study where in chick pea low concentrations inhibiting both root and shoot growth whereas, high concentrations promoted shoot growth. Different plant seedlings respond differently to various IAA concentrations (Sarwar and Frankenberger, 1992).

The current study, at low concentrations, the IAA stimulated above-ground and inhibited below-ground plant variables, however, at higher concentrations the material inhibited all the plant variables. Similar observations on above-ground plant variables were made by Shahab et al. (2009), when mung beans (*Vigna radiata* L.) were exposed to various concentrations of IAA in greenhouse experiments. The finding of the investigation highlighted that low concentrations increased plant height, leaf number, stem diameter and root mass, however, at the highest concentrations the material reduced plant variables (Shahab et al., 2009). Sachdev et al. (2009) reported the effect of IAA on growth of wheat (*Triticum aestivum* L.) under controlled conditions, where almost all IAA concentrations significantly increased plant height and root mass. In the current study, most plant variables plotted against increasing concentrations of IAA had density-dependent growth (DDG) patterns as shown in other plant species (Reetha et al., 2014; Shahab et al., 2009). At high concentrations, IAA inhibited growth, whereas at lower concentrations, it induced filamentation and adhesion (Prusty et al., 2004). Sadak et al. (2013) demonstrated that treating the two faba bean cultivars with 75 mg L^{-1} IAA had positive effects on plant height, number of leaves, fresh and dry shoot mass. Reetha et al. (2014) observed that various IAA concentrations had the efficiency to improve the shoot length, number of leaves and root development of onion (*Allium cepa* L.). Indole acetic acid stimulated plant growth at low concentrations and provided the plant with more branching and larger surface area (Reetha et al., 2014). Additionally, Husen et al. (2017) showed that applying IAA at optimum concentration of 45 mg L^{-1} allowed faba bean to withstand water stress conditions while simultaneously increasing vegetative growth.

Recommended concentrations of IAA on various trees removes large portion of root system, decreases shoot growth, reduces photosynthesis and transpirations and thus hastening senescence of leaves (Arnold and Struve, 1989). Effectiveness of root pruning depends on the type and concentration of plant growth regulator (Liu et al., 2016). Therefore, IAA should not be viewed in isolation, but together with other crop management practices that effectively increase and/or retard root growth (Mohite, 2013). Also, it is important to remember that PGRs are not fertilisers meant to correct a severe nutrient deficiency but are chemicals that in small amounts promote and enhance plant growth through their direct effects on metabolic processes (Gallant, 2004; Bari and Jones, 2009). Using this view, IAA consistently increased number of leaves, plant height and stem diameter at low concentrations, whereas, at high concentrations the material suppressed plant variables.

4. Conclusion

Moringa oleifera plant growth had an IAA concentration-dependent growth patterns over increasing IAA concentrations. Low IAA concentrations increased above-ground plant growth, whereas high concentrations reduced the variable. All tested concentrations of IAA reduced dry root mass and the study demonstrated that these chemicals can be successfully used for pruning roots of *M. oleifera* seedlings to coordinate above-ground plant growth. The integrated mean optimum concentration of IAA on *M. oleifera* seedlings for use in root pruning was established at 1.98 mg mL^{-1} and therefore should be adopted for use on *M. oleifera*.

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