Nursery hardening of *in vitro*-produced banana plants

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Abstract – Introduction. This protocol can be used as a guide for people who wish to construct their own nursery. It describes the methods for receiving, transplanting, managing and hardening young banana tissue culture plants after the weaning stage. The principle, key advantages, starting plant material and time required are presented. **Materials and methods**. This part describes the nursery construction, the nursery media, fertiliser and bags, the handling and transplanting of weaned plants into bags (3 steps), and the management schedule for hardening nursery plants (8 steps). Possible troubleshooting is listed. **Results**. All the characteristics of nursery-hardened plants in bags, ready for transplanting to the field, are formulated.

South Africa / Spain / *Musa* sp. / methods / vitroplants / hardening

Endurcissement en pépinière de vitroplants de bananier.

Résumé – Introduction. Ce protocole peut être utilisé comme un guide pour les personnes qui souhaitent construire leur propre pépinière. Il décrit les méthodes pour recevoir, transplanter, contrôler et endurcir de jeunes bananiers issus de la culture de tissu après sevrage des plants. Le principe, les principaux avantages de la méthode, le matériel végétal de départ et le temps requis sont présentés. **Matériel et méthodes**. Cette partie décrit la construction de pépinière, les milieux, engrais et sacs utilisés en pépinière, la manipulation et la transplantation des plants sevrés conditionnés en sacs (3 étapes), le programme de gestion pour endurcir les plantes en pépinière (8 étapes). Des problèmes éventuels sont énumérés. **Résultats**. Toutes les caractéristiques des plantes endurcies en pépinière, conditionnées en sacs et prêtes à être transplantées au champ sont énoncées.

Afrique du Sud / Espagne / *Musa* sp. / méthode / vitroplant / durcissement

1. Introduction

This protocol can be used as a guide for research centres, farmers or nurserymen who wish to construct their own nursery. It describes the methods for receiving, transplanting, managing and hardening young banana tissue culture plants after the weaning stage [1].

Application

The protocol aims at promoting the acclimatisation and protection of rooted *in vitro* banana plants between rooting in the laboratory and their establishment in a nursery.

Principle

As in the case of weaning, the important requirement for successful hardening is to protect the young plants from environmental stress as much as possible under a net structure. Additionally, careful and dedicated management should be applied in the nursery. All the cultural factors highlighted here need to be optimised in order to produce uniform, healthy, vigorous, and mutation-free banana plants.

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Key advantages

The key advantage of this protocol is that it offers an intermediate level of protection to banana plants in bags, following their intensive protection provided in the weaning house, and in preparation for the unprotected external environment of the open field.

Starting material

This protocol uses young banana plants of 5–12-cm height, arriving from the weaning house [1]. These plants are first extracted from the weaning trays and size-graded by the weaning manager, after which they are packed and delivered to the nursery bare-rooted in polystyrene or cardboard cartons, ready for transplanting into bags (*figure 1*).

Time required

The time taken from transplanting of the young banana plants to their removal from the nursery will be about (6 to 12) weeks, depending mainly on prevailing temperatures, but also on pot size, management level and the growth stage at which the plants are required for the field.

Figure 1.
Weaned plants extracted from trays and placed neatly in a polystyrene box for transfer to the nursery.

2. Materials and methods

Nursery construction

Before the young plants arrive, a strong and durable nursery covered entirely with shade cloth should be prepared. The following aspects of nursery construction are important:

– The structure has to be built with a metal frame or treated wooden poles. Ensure it is about 3 m high, to facilitate easy access and movement inside.
Note: the size of the nursery depends on the number of plants to be hardened in a season. For 80 000 plants, a nursery of 4,500 m^2 is required, based on 70% of the total space occupied by plants and 25 plants·m^{-2} in the planted area. The other 30% of the space is occupied by the central road, side paths and perimeter path.

Use polypropylene shade netting which is either white to reflect heat (in hot areas) or black to absorb heat (in cooler areas). Secure the net as tightly as possible to the metal framework or to the structure of wires and poles to prevent it from tearing. Sew the netting onto tensioned wire supports using nylon thread (*figure 2*).

Inside the nursery, raise the beds above soil level by using granite stones, breezeblock bricks, wire mesh or earth ridges (approximately 1 m wide), which are covered with black plastic sheeting (*figure 3*). Whatever the method used, make sure there is good drainage under the bags, but no contact between the bag and soil. Make the alleys wide enough to allow easy access between the beds. Do not make the beds themselves too wide, otherwise access to plants in the middle of the bed is impeded (*e.g.,* use 1-m-wide beds with 5 or 6 lines of plants).
Note: raised beds are necessary to facilitate handling of plants and to prevent banana roots from penetrating underlying soil which may carry nematodes or pathogens such as *Pythium*.

– For shading, ensure that there is not more than 50% shade inside the nursery at ground level.
Note: forty per cent white shade net gives 15% shade on the ground, whereas 40% black shade net gives 50% shade on the ground. White shade net allows faster growth except for some initial leaf burning. Black net prevents leaf burn but reduces photosynthesis, growth and plant development.

Also cover the sides, ends and corners of the nursery with shade netting to prevent wind driving through the nursery and to avoid full sun on perimeter plants. Make sure the net on the sides and ends is well slanted, and sewn to the ground support wires to divert wind and prevent it from directly buffeting the nursery (*figure 2*).
Note: more sophisticated structures such as a glass greenhouse, with full control of environmental factors, would be better for hardening plants, but much more expensive. The cooling system has to be very effective in this case.

– Hygiene measures to prevent virus transmission and nematode contamination are necessary: locate the nursery at least 1 km away from the nearest *Cucurbitaceae* plants in order to protect the nursery from aphids and possible Cucumber Mosaic Virus infection. Also, destroy all broad-leaf weeds in a wide band around the nursery. Install a footbath containing didecyl dimethyl ammonium chloride, or other approved fungicide, at the entrance to the nursery. Excavate drainage channels inside to lead excess water towards the perimeter.

Prevention of nematode infestation is critical at nursery level if a clean start in the field is to be achieved. Organic composted media would normally be free of pathogenic nematodes, but where soil or river sand is used, there is a risk factor for nematodes. In such a case granular nematicide may be added to the bags as a precaution. The irrigation water should be sourced from a clean borehole or be chemically treated (chlorination) or filtered against nematodes.

Figure 2.
Typical shade net nursery structure with wooden poles braced with crosswires, slanting sides, net sewn onto wires and cement drain along sides.

Figure 3.
Weaned banana plants newly transplanted into the nursery. Note (a) plants raised above soil level on a metal frame, which in turn rests on a polyethylene ground cover, (b) the use of 1.5-L disposable polyethylene bags, (c) uniformly spaced plants in double rows for mutual support, and at a density of 24 plants·m^{-2}.

Nursery media, fertiliser and bags

Various types of organic nursery media can be used including composted pine bark, coconut husk fibre, composted coffee husks, rice husks or milled maize cobs. Use any of these media either alone or mixed with a heavier inorganic component such as soil or river sand. Avoid media mixes containing more than 50% soil or river sand because these components can cause compaction, which increases the risk of waterlogging and root dieback following over-irrigation (such media have high water-holding capacity but low air-filled porosity).

If using a slow-release fertiliser (SRF), premix the basic medium in bulk together with granules of a suitable SRF. Mix the constituents thoroughly at a rate determined by the NPK ratio of the SRF, the duration of the nutrient release period, and the expected duration of the nursery stage. The usual concentration range is 2–4 g SRF·L^{-1} of basic medium (2–4 kg·m^{-3}) [2]. This is based on a 70-day nutrient release period, a NPK ratio of [2:1:2], and an 8-week nursery stage. Alternatively, cheaper fertilising options are to apply granules of quick-release, soluble chemical fertilisers, or solutions via irrigation water (fertigation). In these options, the total nutrient requirement is split several times and small quantities applied frequently during the nursery-hardening period (see also step 6 below).

Note: for bulk mixing SRF, a concrete mixer is much more effective than hand mixing the components on the floor, which can create concentration discrepancies, leading to variable plant growth. Alternatively, slow-release fertiliser can be accurately measured and sprinkled on top of the medium after filling the bags, and worked in before planting.

Ensure the organic component is well composted and the pH is tested. If the pH is below 5, add 2 kg·m^{-3} dolomitic lime during the mixing process. The potting medium must have the following characteristics:

– It must be composted, sterilised, pasteurised and watered with chlorinated water during the entire nursery period.

– It must be watered immediately before delivering the plant to the field grower.

– It must be firm and with no tendency to collapse after removing the plant from the bag.

– Water-holding capacity and air-filled porosity characteristics should be similar to those proposed for weaning media [1].

If the nursery medium is not composted (organic) or sterilised (sand/soil), and the nursery cannot be irrigated with chlorinated water, then drench the filled bags with an approved fungicide as a precaution against soil diseases (*e.g.,* propamocarb hydrochloride or mancozeb/metalaxyl-m at the recommended concentration). Use either thin-gauge disposable black plastic bags (*figures 3, 4*) or thick re-usable polypropylene pots ('polypots') for growing out the young plants on raised beds (*figure 5*). Common sizes for nursery bags or pots are 1.5–2.5 L (average 2 L [3]), although much smaller pots are used in Taiwan [4]. Bags or pots must contain adequate drainage holes.

Note: the advantages of a larger 2.5-L bag are that spacing of plants can be wider in the nursery, plants can be left for longer in the bag, thus allowing them to harden more, and off-types can be more easily detected on larger nursery plants. However, these plants have to be sold at a higher price due to increased bag, medium, fertiliser and transport costs, and fewer plants fitting into the available nursery space.

Figure 4.
Removal of weaned plants from polystyrene boxes and transplanting into nursery polypots. Note plug must be placed 2 cm below surface of medium.

Handling and transplanting weaned plants into bags

• Step 1

Fill the nursery bags to the brim with the pre-mixed potting medium prior to arrival of the plants from the weaning house, in order to prevent unnecessary delay before planting. *Note*: there are three reasons for filling the bags full as recommended. Firstly, the bags and plants are better stabilised against each other on the base structure; secondly, this prevents the sides of the bags from collapsing inwards which would reduce water access into the bag; thirdly, rooting volume is maximised.

• Step 2

Count and closely inspect all plants on arrival at the nursery and immediately refer any queries or problems to the supplying laboratory. If there has to be a delay between arrival of the boxes and planting out, do not allow the boxes to overheat or plants to dry out, but store them with lids on in a cool place between 14 °C and 20 °C.

Mature weaned plants [(5 to 12) cm] from the seedling trays in the weaning house are extracted and then transferred to the nursery in cardboard boxes (short distance to nursery) or polystyrene boxes (long distance to nursery). After extraction from the trays, size-grade the plants and place similar-sized plants together in the same box. Layer the plants carefully in rows between moist sheets of tissue paper, taking care not to damage the leaves. Label the box with cultivar name, size grade and destination nursery. These tender plants with open root plugs can survive in a healthy condition for 5–7 days in a cooled box, but the sooner they can be planted out after packing, the more successful the transplanting will be.

• Step 3

Water the medium in the bags well before planting out. This facilitates planting and ensures the young transplants do not dry out. Make a hole slightly larger and deeper than the plug size in the middle of the medium surface, using a conical or pyramid-shaped tool depending on the plug shape. When planting, make sure the hole is deep enough for the plug plus 2 cm of stem base to be covered. Do not cover the lower leaves themselves. After inserting the plant, fill in the hole and compact it only lightly. Water the bags thoroughly again after transplanting.

Note: deep planting encourages plant strength and stability, whereas shallow planting can cause plants to become unstable during later movement and transport.

Figure 5.
Ideal morphology and plant characteristics of Williams banana plants in 4-L disposable bags, at the end of the nursery hardening period.

Management schedule for hardening nursery plants

• Step 1. Size-grading

The young plants should be size-graded by the nurseryman before planting into nursery bags. Thus, only similar-sized plants will be planted on each raised bed to enable uniform development of the plants and to minimise variability (*figure 5*).

Note: size-grade the plants once more prior to dispatch for planting in the field.

• Step 2. Labelling

Label the nursery beds accurately according to cultivar, date of planting, origin and destination.

• Step 3. Plant density

Maintain a plant density of about 25 plants·m^{-2} (excluding pathways but including any double row gaps) (*figure 3*). This is considered to be the optimum for growing out banana plants in a nursery.

However, to achieve maximum cost-effectiveness in the nursery, establish plants at a density of 30 plants·m^{-2} and, for maximum individual growth potential, establish plants at a lower density of 12 plants·m^{-2}, which gives large, sturdy plants at maturity [2].

Note: A population density lower than 20 plants·m^{-2} generally wastes nursery space and increases the growing and transport costs per plant. Conversely, a density of more than 30 plants·m^{-2} creates excessive plant-to-plant competition which, in turn, causes elongation, etiolating and premature leaf dieback. Such plants may establish poorly in the field. Other disadvantages of high nursery density are that (a) water penetration through the foliage is restricted, causing uneven growth; (b) identification and culling of off-types is more difficult; (c) counting of plants for stock control is also more difficult.

• Step 4. Spacing

When strong, re-usable 'polypots' are being used, space the plants equidistant from each other for equal light distribution and watering efficiency (*figure 6*). However, if collapsible bags are being used, space the plants in double rows since these bags can easily fall over and the double row arrangement gives mutual bag support plus additional light on one side of the plant (*figure 3*).

Figure 6.
Uniform and accurate irrigation of nursery plants using a "spaghetti" drip system (one dripper to each bag).

Note: Do not, under any circumstances, establish nursery plants at random with variable spacings, because this encourages uneven competition and variable growth.

• Step 5. Watering schedule

If using a light 100% organic medium, water the banana plants at least once a day and up to three times a day if the weather is hot and plants are large [5]. If a heavier medium is used, water less frequently but test wetness by hand to determine when to water. When watering by hand with a hosepipe and rose on the end, leaves can be wetted as needed and the bags also receive water. For uniform watering of bags, a spaghetti dripper system is optimal since each bag receives exactly the same amount of water (*figure 5*). However, this system is expensive and leaves cannot be wetted and cooled when it is hot. An overhead microspinner system cools the leaves regularly and alleviates stress but penetration of irrigation water to the bags may be variable due to leaf interception (*figure 5*). A dripper and microspinner together is the ideal nursery combination, but this technique is very expensive.

Note 1: a lighter medium must be watered more often but has a lower risk of waterlogging due to the high air-filled porosity. Overwatering of a heavy medium, however, can easily cause waterlogging and root dieback due to the high water-holding capacity and low air-filled porosity. Such damage can be identified externally by wilted leaves, leaf yellowing, lack of new leaves and leaf edge necrosis. Internally, damage is seen by grey/brown discoloured roots inside the bag, especially at the bottom.

Note 2: plants growing under cooler, subtropical winter conditions require much less water due to the lower evaporation rate and the reduced extraction capacity of the roots. It is a common fault to overwater under such conditions, which predisposes the plants to waterlogging and root dieback.

Note 3: plants should never experience water stress, either from too little or too much water.

• Step 6. Fertiliser schedule

If using a slow-release fertiliser, the initial application may be enough for the nursery lifetime of the plant. Alternatively, soluble

granular fertilisers or nutrient solutions (fertigation) can be used, but ensure that these are applied on a 'little and often' basis, due to their high solubility and the risk of burning. Vuylsteke applied urea [(0.1 to 0.5) g] and potassium chloride [(0.2 to 1.0) g], dissolved together in 100 mL water per plant at 4-week intervals [6]. However, the plants have to be 3–4 weeks old before any such fertiliser programme is initiated. Soluble fertilisers can easily cause plant burn (leaf marginal necrosis) if applied to excess. Israeli *et al.* recommend that the concentration of nitrogen in any solution of fertiliser diluted with irrigation water (fertigation) should not exceed 60–100 $mg \cdot L^{-1}$ nitrogen on young plants and 120–150 $mg \cdot L^{-1}$ on larger plants [2]. However, if leaves become pale in colour in the nursery, apply weekly sprays with a recommended foliar feed containing micronutrients and nitrogen.

• Step 7. Pest and disease management

Although pests and diseases are not usually a problem in the nursery, it may sometimes be necessary to initiate a spray programme, using currently recommended chemicals, for looper caterpillar, red spider mite, fungal leaf spot or *Pythium* fungus (see also under troubleshooting below).

• Step 8. Mutation and off-type management

The nurseryman must be very severe about culling mutations, off-types and weak-looking plants during nursery hardening, and especially at the stage when plants are being selected for the field. The larger the plants in the nursery, the easier it becomes to detect mutations. Common mutations [2, 7–10] are mentioned below and should be eliminated in the nursery:

– mosaic/variegated: yellow/white streaks or blocks on the leaf surface: easy to detect even on small plants;

– masada: irregular translucent or black blotches and Vaseline-like stripes on the leaf surface, especially the undersurface: easily seen when leaves are held up to the light;

– narrow and misshapen leaves: some have a wavy, crinkly appearance, with indented margins;

– leathery leaves: leaves have an unnatural thick, leathery feel;

– lack of red anthocyanin pigment in the leaves: easy to detect at a young stage;

– dwarf mutation: plants are shorter than normal; leaves are shorter and wider than normal; internodes are shorter and petioles are shorter and curl around more. Thus, all smaller, weaker-looking plants with short, wide leaves should be culled, and this is especially important in the beds where plants were size-graded as being the smallest in the batch.

Note: dwarfs are usually the most common mutation in the field because they are sometimes difficult to detect in the nursery and often pass through strict nursery culling. Usually they are only confirmed in the field.

A tissue culture laboratory should supply up to 5% extra plants with all orders of weaned plants. This amount allows the nurseryman to select out and replace all off-types and weak plants in the nursery plus allowing him to establish 2% extra plants in bags for the grower client. These plants are to be used as replacements if off-types are discovered in the field 1 or 2 months after planting.

Troubleshooting

Many problems can occur in the hardening nursery:

(a) White bleaching can appear on the young leaves after transplanting from weaning. Cause: heat stress and sunburn in the new, harsher environment.

Solution: frequent misting; use black shade net for the first 3 weeks in the nursery; time for plants to settle and produce new leaves.

(b) Leaves show uniform lines of holes. Cause: looper caterpillar *(Chrysodeixis acuta)*.

Solution: spray bacterial-based insecticides down the central funnel at the recommended dosage.

(c) Older leaves turn orange-brown in colour, minute insects on underside. Cause: red spider mite.

Solution: remove infected leaves; spray with an acaricide such as abamectin or bromopropylate at the recommended dosage.

(d) Round, pea-sized leaf spots on older leaves are observed in late summer/autumn. Cause: leaf fungal infection under hot conditions (various fungi).

Solution: spray when conditions are hot and humid with systemic fungicide such as propiconazole, at the recommended dosage. Remove leaves showing symptoms.

(e) The youngest leaves wilt and turn brown; a red/brown ring in the rhizome when cut (*figure 7*). Cause: *Pythium* soil-borne fungal infection.

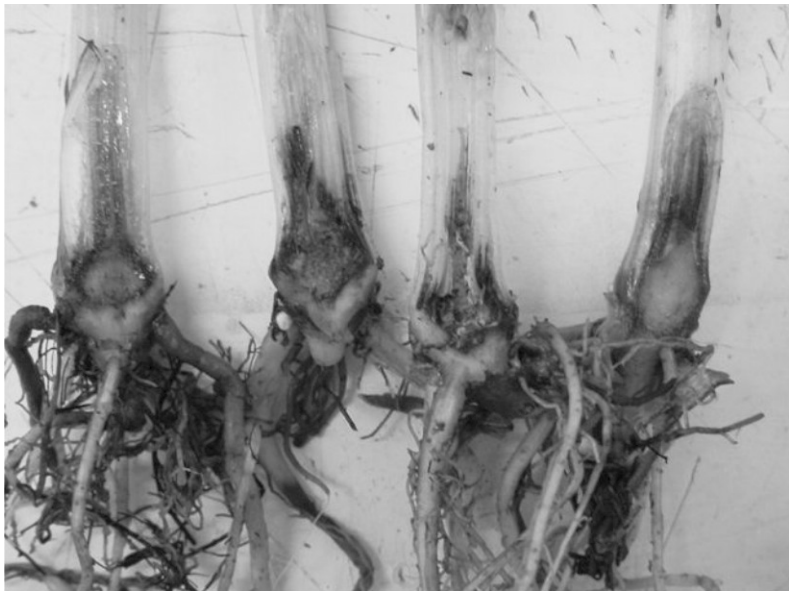

Figure 7.
Nursery banana plants cut open to expose a red/brown ring in the rhizome, indicating *Pythium* (or other pathogenic fungal) infection.

Figure 8.
Young transplants in the nursery with leaves which have wilted, folded and turned yellow/brown, and with an absence of new roots. This can result from over-irrigation and waterlogging.

Solution: use composted or sterilised medium; use chlorinated water; drench bags with fungicide as described under nursery media.

(f) Plants present leaves folded and wilting, and turning yellow/brown. Roots turning grey/black and lack healthy side roots (*figure 8*). Cause: waterlogging of medium due to over-irrigation and/or poor drainage.
Solution: rectify the irrigation schedule; use lighter medium.

(g) Leaves are small and narrow; and have wavy leaf margins; short internodes; no new growth. Cause: night temperatures below 11 °C causing chilling symptoms and growth cessation (only in subtropical locations).
Solution: plant in the nursery 2 months before the start of winter if plants are needed in early spring; plant after winter for plants needed in late spring. Avoid having young transplants in the nursery during winter months.

(h) Leaves are smaller in size and light green or pale yellow in colour (*figure 9*). Cause: nitrogen and/or trace element deficiencies.
Solution: increase the initial rate of slow-release fertiliser; apply weekly foliar sprays of a recommended product containing nitrogen and trace elements.

(i) Leaves show yellow/white streaks, irregular blotches, an indented margin, or lack of anthocyanin Cause: various forms of mutation (see step 8 above).
Solution: remove and destroy the affected plants.

(j) Plants are small, stunted and yellow; they do not grow even under optimal climatic conditions. Cause: acidic medium with very low pH.
Solution: plant out into the field even if plants are too small. This problem cannot be rectified in the bag. For new medium, check pH and add dolomitic lime if necessary.

(k) Plants are somewhat stunted with a layer of necrotic circles around the leaf margins. Cause: saline irrigation water.
Solution: change the irrigation water to a new source and have it analysed.

(l) Plants are growing at variable rates in the nursery, causing poor uniformity (*figure 10*). Cause: poor size-grading at planting; variable spacing of plants; overcrowding.
Solution: size-grade the plants accurately at planting; space plants equidistant from each other.

3. Typical results obtained

Nursery-hardened plants in bags, which are ready for transplanting to the field, should have the following characteristics in medium-sized Cavendish dessert cultivars ('Williams' or 'Grand Nain') [11].

– The height of the plants measured from soil level to the junction of the two youngest leaves should range from 20 cm to 30 cm in a (1- to 2-) L bag and from 30 cm to 50 cm in a 2.5-L or larger bag.

– The ratio of the plant height (from soil level to the junction of the two youngest leaves) to the base diameter (at soil level) should be a maximum of [10:1]. More than this is an indication that the plant has grown under excessively crowded conditions in the nursery and has become etiolated.

At the time of dispatch, nursery plants are expected to have the following characteristics (*figure 4*):

– Plants are firm and stable in the bag and the pseudostem is not wobbly at soil level.

– Bags are "solid" when squeezed, indicating complete ramification of the potting medium by roots. Individual primary roots can be felt pushing against the bag. A soft bag indicates inadequate root development. A bag which has become pot-bound with roots is not necessarily a disadvantage, whereas a soft bag containing an underdeveloped root system is a distinct disadvantage.

– The pseudostem is not tall, thin and etiolated, but within the proportions described above. The base of the pseudostem has a red or dark green colour rather than a light green or yellow colour.

– All leaves along the pseudostem have a dark green, healthy and normal appearance, and have the normal proportion of anthocyanin pigmentation in the lamina. Leaves are not pale green, misshapen or wilted at delivery. In particular, leaves are not yellow or senescent in any way.

– Leaves are equally spaced along the pseudostem with wide internodes and not rosetted at the top of a bare pseudostem.

Figure 9.
Fertiliser trial showing nursery plants with low nitrogen (left), medium nitrogen (middle) and high nitrogen (right). Sufficient nitrogen is critical for banana nursery growth.

Figure 10.
Variable growth rate of banana plants in the nursery due to (a) overcrowding at high density and/or (b) poor size-grading at planting.

– There is a minimum of five healthy green leaves on a plant ready for the field.

– All plants in a batch ready for the field are uniformly sized and do not vary in height by more than 10 cm from smallest to largest.

– Plants show no signs of zinc deficiency (pink tinge on underside of lamina), nitrogen deficiency (pale green leaves with pink margins on petioles), cold damage (small, thin lamina with wavy or necrotic edges), heat damage (white-bleached patches or brown necrotic spots on the edge of the lamina), fungal infection (round, necrotic spots with premature yellowing of the lamina), looper caterpillar damage (uniform lines of holes across the lamina), mite damage (speckling on the underside of the lamina with a fine web and visible red mites), or any other visible symptoms of deficiency or pest and disease attack.

– Plants delivered should show no visible signs of abnormality or mutation as described under the management schedule.

– The medium must not disintegrate from the root ball when the bag is removed.

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