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Managing Coffee Nematode Decline

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This publication is a collection of information developed for workshops intended to inform coffee growers about the threat of the plant disease known as coffee nematode decline. For the convenience of the coffee growers, various workshop handouts are brought together here in one place.

What is coffee nematode decline?

It is a severe disease of coffee trees in the Kona region of the Big Island. Severely affected trees are stunted and unthrifty, have thin and wobbly stems, are easily uprooted by hand, have dying or declining branches, have leaves that are yellowing, brown and/or wilting, and have roots that are galled, swollen, corky, and rotten.

What is the cause of the disease?

Kona coffee nematode decline is caused by a plant-parasitic nematode known as *Meloidogyne konaensis*, the Kona coffee root-knot nematode.

What is a nematode?

A nematode is a roundworm that lives in water or in soil, often as a parasite of plants and animals. Plant-parasitic nematodes are microscopic in size (300–1000 mm long by 15–35 mm wide) and attack roots of many plant species in Hawaii. One general class of nematodes is referred to as "root-knot" nematodes. *Meloidogyne konaensis*, the Kona coffee root knot nematode, is a type of root-knot nematode.

What is "root-knot"?

"Root-knot" is another name of the disease that is caused by the feeding activities of root-knot nematodes. Affected roots appear knobby, galled, lumpy, or swollen.

How do root-knot nematodes cause disease?

Root-knot nematodes damage the plant tissues that conduct water and nutrients from roots to the actively growing parts of the plant. This damage results in yellowing of leaves, wilting of foliage, and ultimately plant death. The physical and physiological damage nematodes do to root systems causes the roots to swell and crack.

What is the life cycle of the root-knot nematode?

Root-knot nematodes (*Meloidogyne* species) have a six-stage life cycle: the egg stage, four juvenile stages, and the adult stage (male and female); see p. 2. On coffee, this life cycle is completed in about 6 weeks. The life cycle begins when freshly hatched root-knot nematode juveniles penetrate coffee root tips and move deep within the root to a position near the root vascular system. Here, the nematodes establish permanent feeding sites. While feeding, the juveniles swell. Most of them become pear-shaped adult females; a few change to become vermiform males. Their feeding causes an infection in which the plant is forced to produce very large root cells (called "giant cells") that nurse the nematodes. The roots also are stimulated to produce abnormally large cells and also to increase the number of root cells around the nematodes, which creates the swollen, gall-like symptoms of the disease. A hundred or so eggs are laid by an individual mature female within the roots or at the root surface. Upon hatching, the emerging juveniles may re-infect the same root or neighboring roots and start the disease cycle over again. In severe infections, there can be many thousands of nematodes feeding in each gram of root tissue.

How do root-knot nematodes survive in the soil?

They can survive for months to a few years as eggs or juveniles in the soil or within root fragments in the soil.

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How are root-knot nematodes spread to or within my farm?

They are primarily spread by water, machinery, animals, and humans. Planting of infected seedlings into uninfested soil is one of the most common and effective ways these pests are dispersed. They move very short distances (only an inch or so) under their own power.

Can root-knot nematodes be eliminated from coffee farms?

Unfortunately, it is very unlikely that these pests can be totally eliminated from an infested farm, due to their great numbers and their ability to survive adverse conditions. The best way to deal with them is to try to continually reduce their population numbers to levels that do not cause great damage. This is accomplished by finding ways to interrupt the nematode life cycle, or to slow it down.

Life cycle of the Kona coffee root-knot nematode

The life cycle of the Kona coffee root-knot nematode is completed within approximately 6 weeks on coffee (vs. 3 weeks on tomato). Therefore, several generations of new nematodes can be produced within one year. The life cycle begins when juveniles are formed within eggs laid by an adult female. The adult females are often embedded deep within the root system, feeding continually. The second-stage (stage II) juveniles hatch from the eggs and move through the soil to a root tip, which they penetrate to establish a feeding site. This feeding induces "giant cells" to form.

The juveniles grow and mature through several stages, eventually becoming the typical swollen, sedentary, pear-shaped adult females. Some of the juveniles become males that leave the root. The males do not feed or cause disease.

Each female can lay up to 500 or more eggs (either within the root or at the root surface), but they normally produce about 100–200 eggs. She will produce eggs for a few days, then die.

General disease cycle for most species of root-knot nematodes

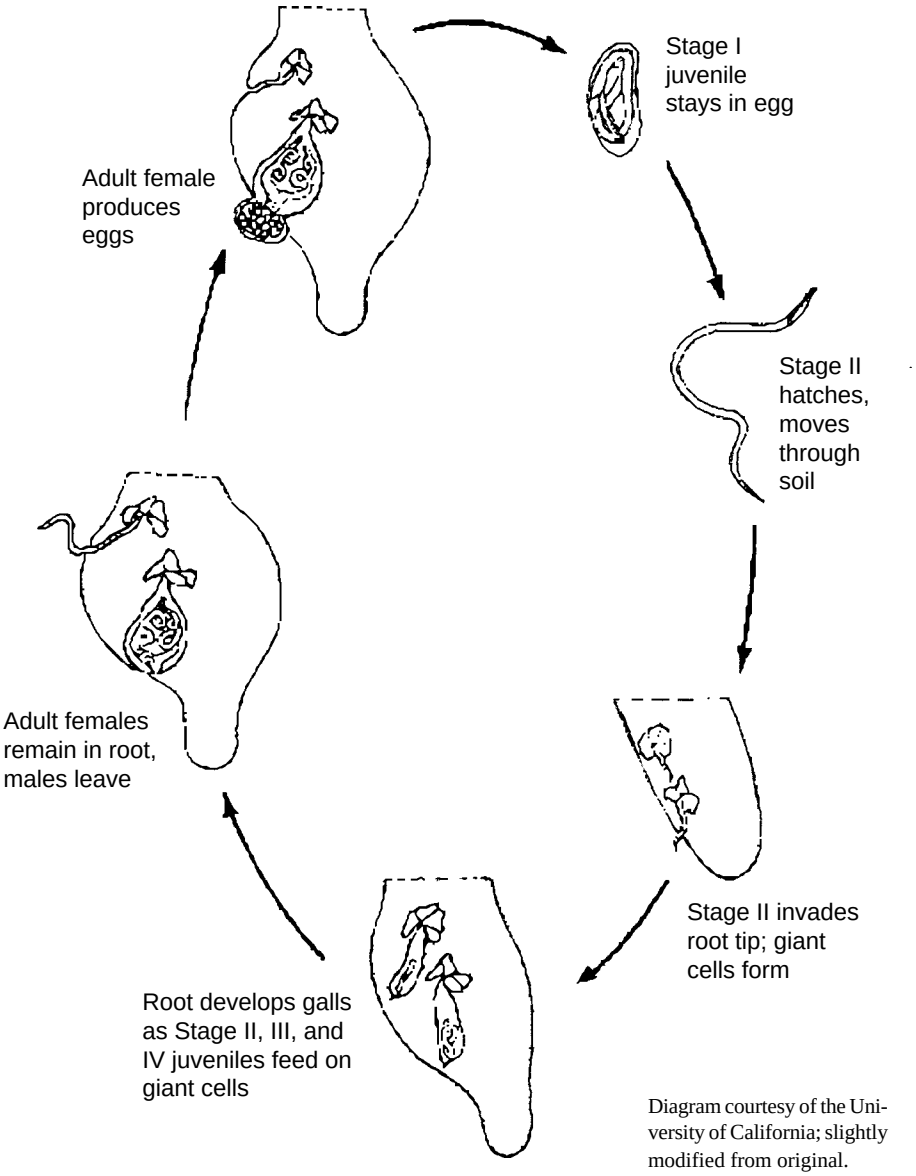

Diagram courtesy of the University of California; slightly modified from original.

How severely is my farm affected by coffee nematode decline?

Use the following checklist to estimate the severity of nematode decline on individual plants. If you check off one or more symptoms in each category, there may be a disease problem occurring or developing.

Leaves
- ❏ Leaves wilting and drooping ("flagging")
- ❏ Leaves yellowing
- ❏ Leaves brown, falling off tree

Branches
- ❏ Some branches have thin foliage
- ❏ Some branches dying back

Fruits
- ❏ Change from green to raisin
- ❏ Large amount of fruit drop

Stem
- ❏ Stem narrow or thin
- ❏ Stem wobbly
- ❏ Plant stunted
- ❏ Plant easily uprooted by hand

Roots
- ❏ Roots swollen and galled
- ❏ Roots appear corky
- ❏ Tap root destroyed or non-functional
- ❏ Few secondary or feeder roots
- ❏ Roots discolored, rotten

Now, use the table below to estimate the severity of coffee nematode decline on your farm. *Please confirm your diagnosis by sending soil and root samples for nematode assay to the CTAHR Agricultural Diagnostic Service Center via your local CTAHR Cooperative Extension Service office*.

Severity	***Foliar symptoms***	***Root symptoms***	***Field symptoms***
No disease	Leaves dark green, not flagging, no dieback of branches or defoliation; thick stem diameter; plants strongly rooted (hard to wobble).	Extensive feeder root system, healthy tap root and feeder roots, white in color, no galls or terminal swellings visible.	Trees have full canopy, no areas in field are showing decline or stress.
Moderate disease	Some leaf flagging; some leaves are yellowing, some dying (necrosis); some defoliation and/or branch dieback; some plants have wobbly stems and a relatively thin stem diameter; some plants appear to be stunted; flowering appears to be somewhat hindered.	Galling and terminal swellings visible on fine roots and secondary feeder roots; some root discoloration is evident, relatively low number of fine feeder roots; some galling and swelling on primary roots (tap root).	Localized or patchy areas of declining trees in the field.
Severe disease	Extensive leaf yellowing and/or severe leaf flagging, along with much leaf necrosis, leaf drop, and branch die-back; very thin stem diameter or very wobbly stems; very poor flowering; severe plant stunting; fruits turn to raisins and drop; tree dies.	Severe galling and swelling of primary root system; tap roots and secondary roots heavily cracked and "corky" in appearance; extensive root necrosis; missing or heavily damaged tap root; feeder root system virtually absent.	Many trees have drooping, yellow, and dead leaves; some areas of the farm have severely diseased and declining or dead trees.

Myths and truths about the Kona coffee root-knot nematode

Much "general knowledge" about coffee cultivation circulates among coffee producers. Some of this knowledge is "tried and true," but some of it is based neither in common-sense logic or scientific principles. Since nematodes are subterranean and virtually invisible, they have often been ignored as a cause of disease.

Common *myths* in coffee production

- Nematodes are not a problem on coffee.
- Fertilizer will overcome nematode damage.
- Nematode-injured plants require more water.
- "Natural nematicides" will eliminate nematodes on coffee.
- *Pulapula* are a good source of planting material.

The *reality* of the disease threat from root-knot nematodes

- Several nematodes are causing significant loss of coffee yield:

  Root-knot nematodes
  Lesion nematodes
  Burrowing nematodes

The major problem of coffee in Kona is decline caused by the Kona coffee root-knot nematode. It occurs on about 85% of the acreage in the Kona area. On infested coffee farms, the overall yield loss is estimated at 60%.

Fertilizers may actually increase injury to nematode-infected roots, or may simply be wasted.

The nematodes cause serious root injury. The injured roots are more susceptible to other injury, especially from inorganic fertilizers, because they are formulated as salts and may damage injured roots. In addition, root uptake of nutrients is reduced, so the fertilizer will probably be leached out of the soil and not absorbed by the diseased roots. Proper fertilizer use may mask the presence of root-knot disease for some time while extending the productive life of a diseased plant. However, fertilizers are not able to cure the infections caused by the nematodes or prevent the nematodes from reproducing. So, in the long run, fertilizers actually enhance nematode populations and delay the inevitable, which is that severe disease will eventually take its toll on a heavily infected plant.

Plants wilting due to nematodes may not be wilting because of water deficit.

Nematode-injured roots are very inefficient in water uptake. Thus, even though water is adequate, the roots do not take up enough water to meet the demands of the plant. Consequently, wilting occurs. Additional water to compensate for the poor uptake often creates a root environment that is low in oxygen. This causes more root damage and may speed the death of the plant.

Organic amendments are beneficial but do not adequately control nematodes.

Many organic compounds have been placed on the market over the years. They are sold as a "cure" based on various claims. Many of these products have some beneficial effects, similar to applying manure or compost. Clearly, the addition of several tons of organic matter per acre is beneficial in reducing nematode populations, but as a single practice, it does not adequately reduce the number of nematodes to be of economic benefit. Any product sold with promises that appear too good to be true should be questioned.

Pulapula *(volunteer coffee seedlings) are often infected with nematodes.*

Infected *pulapula* are a major reason that nematodes are spread and new infestations become established. It is best to germinate seeds and grow the seedlings in sterile media. If the farm is already infested, use resistant rootstocks, because planting healthy "Kona typica" seedlings into nematode-infested soil will result in disease soon after planting.

Coffee nematode decline disease management options

Management objective:
To reduce populations of nematodes to non-damaging levels.

Preplanting
Bare-fallow severely infested fields for at least 9–12 months before replanting. Keep the field free of weeds. This will eliminate the nematodes' host reproduction sites within the field. Alternatively, grow a cover crop (e.g., sunnhemp) that is not a nematode host, or one that is antagonistic to nematodes (e.g., French marigold); however, these practices will not be optimally effective if there is any weed infestation in the cover crop.

Planting material
IF the farm is free of the Kona coffee root-knot nematode, plant healthy, vigorous seedlings only. Eliminate all infected seedlings from nursery operations. Grow coffee seedlings only in sterile media, and carefully follow nursery sanitation practices. Do not use *pulapula* plants for replanting. Do not plant infected seedlings. *IF* the farm is infested, consider using nematode-resistant rootstocks.

Weeds
Control all weed hosts of the root-knot nematodes within the field, especially in areas surrounding coffee trees and between rows. Eliminate all coffee *pulapula* growing in the field. Coffee *pulapula* can harbor very large populations of root-knot nematodes.

Replanting
Replant heavily infested fields with grafted plants on rootstock that is resistant to the nematodes. If possible, replant higher-elevation areas of your farm first, and work your way down-slope to minimize movement of the nematodes via soil erosion.

Soil management
Incorporate organic matter into the soil near coffee trees to stimulate microbial competition for the nematodes and to improve soil characteristics. Organic mulch also helps to retain soil moisture and provide some nutrition for the coffee plants. Examples of mulches include coffee pulp and parchment. Keep the mulch several inches away from the coffee stem.

Sanitation
Avoid introducing nematodes from uninfested areas to infested areas.

Monitoring
Know your farm. Do a detailed sampling of your farm for nematode assay so that you can identify any areas of heavy nematode infestation. Survey your farm regularly to estimate the distribution and severity of decline symptoms. Keep detailed records of your observations.

Cultural practices
Avoid over-irrigation and over-fertilization in heavily diseased or nematode-infested areas. Over-irrigation enhances root rot and favors nematode reproduction and movement within the soil. Over-fertilization may also promote root rot through salt injury to root tissues.

Minimize plant stress due to other factors (insects, weeds, etc).

Get frequent soil and leaf tissue analyses done to assess crop nutrient status. Nematode-infected coffee trees typically have nutrient imbalances.

A recommended coffee root-knot nematode management program for nematode-infested farms

Remove sick trees

Remove all trees and as many roots as is feasible from the infested field. Prompt removal is important to prevent nematode population growth.

Field drainage pattern

Grade fields to have a drainage pattern that minimizes erosion *and* minimizes movement of nematodes through the field.

Fallow

Maintain the area in a fallow condition until nematode populations have been decreased to non-detectable levels. Some practices that may help reduce the nematode populations more rapidly are

- Keep the field free of weeds that may harbor nematodes: e.g., *pakai* (amaranth).
- Provide conditions to accelerate root decomposition.
- Sample for nematodes during fallow.
  - Soil sampling—Collect soil samples quarterly or semiannually and send to a laboratory for assay.
  - Bioassay—Plant tomato seedlings randomly throughout the farm, grow for 21 days, then carefully remove them from the soil to keep the roots intact. Look for root swellings and determine the amount of swelling.

Replanting

Replant when assays for nematodes indicate that the nematode population is below a level that will cause damage, or replant between old rows. Considerations for replanting:

- Germinate seeds of rootstock (*Coffea dewevrei* cv. Fukunaga) and scion in a nematode-free nursery.
- Graft scions onto rootstocks and transplant the grafted seedlings into a nematode-free medium in 1-gallon containers.
- Transplant grafted trees into the field during the rainy season when the plants are approximately 9 months old.

Field maintenance after replanting

- Remove (mechanically or by hand-pulling) all volunteer coffee seedlings (*pulapula*).
- Plant groundcovers between the rows to provide maximum competition with weeds (remove hilograss and prevent its establishment).
- Use herbicides only in extreme cases.
- Optimize fertilizer efficiency based on soil and foliar tissue analyses.
- Periodically monitor the field (an annual assay is recommended)

Monitoring

Use both a soil assay and a bioassay. If nematodes are detected at higher populations than expected, consult the UH-CTAHR Cooperative Extension Service faculty.

Status of crop plants as host of the Kona coffee root-knot nematode

Genus name	Common name	Cultivar examples, or plant type
Good hosts		
These plants can support rapid nematode reproduction and very high nematode populations. They should not be grown in or near coffee fields.		
Brassica	Broccoli, cabbage	Chinese broccoli, Chinese pak-choi, Michihili Chinese cabbage, green mustard cabbage, others
Capsicum	Pepper	California Wonder, Keystone
Cucumis	Cantaloupe, cucumber	Hybrid Lani, Oriental Explorer, Space Master, etc.
Cucurbita	Pumpkin	Early Sugar, Pie
Daucus	Carrot	Lady Finger
Eleocharis	Water chestnut	–
Hordeum	Barley	Perinco, Boone
Lycopersicon	Tomato	Bounty, many others.
Pisum	Pea	Manoa Sugar
Raphanum	Radish	Daikon, Long
Solanum	Eggplant	Waimanalo Long, Long Purple
Zea	Corn	Hybrid Sweet #10A, others
Zingiber	Ginger	Both edible and ornamental types
Hosts		
These plants can support a moderate amount of nematode reproduction. They should not be grown in or near coffee fields.		
Citrullus	Watermelon	Extra sweet, Sweet Ibuki, Charleston Grey
Ananas	Pineapple	Smooth Cayenne
Glycine	Soybean	Holladay, Ciba 3616
Phaseolus	Pole bean	Hawaiian Wonder, Manoa Wonder
Lactuca	Lettuce	Anuenue
Ipomoea	Sweetpotato	Okinawan
Gardenia	Gardenia	–
Poor hosts or non-hosts		
Arachis	Peanut	–
Avena	Oats	Brooks, NK Coker 716
Chrysanthemum	Chrysanthemum	–
Gossypium	Cotton	Deltapine, KC 380
Crotolaria	Sunnhemp	Tropic Sun

Information from Fengru Zhang and D.P. Schmitt, 1994, "Host status of 32 plant species to *Melodoigyne konaensis*." Supplement to *Journal of Nematology* 26(48):744–748. Refer to the article for more detailed information on these hosts.

Weeds hosts of the Kona coffee root-knot nematode

It is important to keep all potential hosts of *Meloidogyne konaensis* under control within coffee fields.

Scientific name	Common or local name	Host status
Grasses		
Paspalum conjugatum Berg	Hilo grass	Host
Eragrostis grandis. (L.)	Lovegrass, *hakonokono*	Non-host
Panicum maxumum Jacq.	Guinea grass	Non-host
Eleusine indica (L.)	Wire grass, goose grass	Non-host
Broadleaf plants		
Coffee arabica L.	Coffee *pulapula*	Good host
Amaranthus viridis L.	Slender amaranth, slender pakai	Good host
Abutilon grandifolium (Willd). Sweet	Hairy abutilon	Poor host
Galinsoga parviflora Cav.	Fuji grass, galinsoga	Poor host
Bidens pilosa L.	Spanish needle	Non-host
Chamaescyse hirta L.	Garden spurge	Non-host
Emilia fosbergii Nicholson	Tassel flower	Non-host
Portulaca oleracea L.	Pigweed, purslane	Non-host
Sedges		
Cyperus rotundus	Purple nutsedge, nutgrass	Good host

Host status

This refers to the plant's ability to support reproduction and populations of the Kona coffee root-knot nematode. The host status was determined by sampling a nematode-infested coffee field with a history of replant problems. Host status was evaluated by comparing the number of nematodes recovered from the weeds with the numbers of nematodes recovered from the preferred host, coffee seedlings.

Good host	allows nematode reproduction in very high numbers
Host	supports an intermediate amount of nematode reproduction
Poor host	allows only a few nematodes to reproduce
Non-host	immune to infection by the Kona coffee root-knot nematode

Grafting coffee for management of coffee nematode decline—the basics

Grafting the commonly grown arabica-type coffee cultivars (i.e., 'Guatemalan') onto a nematode-resistant, robusta-type rootstock (i.e., CTAHR's "Fukunaga" selection of *Coffea liberica* var. *dewevrei*) may be used to fight root-knot nematodes when replanting into nematode-infested fields or when starting new farms. The cleft grafting method is reliable for obtaining healthy and vigorous grafted seedlings.

Coffee can be multiplied and grown either sexually (through seeds) or asexually (through vegetative propagation). Grafting partially combines both of these approaches. During grafting, a scion with desirable characteristics is joined onto a rootstock plant.

Grafting of coffee has been used in many countries to manage insect pests of coffee and, more recently, to address nematode decline of coffee. In general, the robusta-type coffees have proven to be the most tolerant of plant pests and diseases, and therefore they are often the rootstock of choice for farmers who are battling pests and diseases of coffee.

The rootstock

The rootstock is the plant that provides the root system for the grafted plant for the rest of its life. The rootstock often determines the potential size and vigor of the tree and may impart important disease and pest resistance to the scion without adversely affecting the quality of the fruit that the tree produces. Different rootstocks are used for managing the various nematode pests of coffee, depending on the targeted nematode. In Brazil and Guatemala, for example, *Coffea canephora* is the most common rootstock (it is both nematode-resistant and quite drought hardy).

In Hawaii, *Coffea liberica* var. *dewevrei* has performed well in a long-term experiment in soil infested with the Kona coffee root-knot nematode. The *C. dewevrei* selection named Fukunaga is recommended by the UH-CTAHR for grafting and for planting in nematode-infested fields.

The scion

The scion is the plant that is grafted onto the rootstock. It is the top part of the plant, which produces the coffee beans. Scion varieties must be compatible with rootstocks. Research indicates that *Coffea arabica* cultivars 'Guatemala', 'Catuai', and 'Caturra' are quite compatible with *C. dewevrei*. These arabica coffees have a better cup value than *Coffea robusta* coffees, and thus cultivars of *Coffea arabica* are the preferred scion for *robusta* rootstocks, but not vice-versa.

The cambium layer

The cambium is a thin layer of cells between the bark and the woody tissue of the coffee plant stem. This layer undergoes continuous cell division and multiplication and produces new wood and bark as the tree grows. The cambium layer is usually a green color. *Successful grafting depends on lining up the cambium layers of the scion and rootstock and then providing conditions that enhance healing.* When grafting coffee cultivars, it is important to get the cambium layers of both the rootstock and the scion stems in direct contact as quickly as possible, so that when the cells start dividing the union heals quickly. To achieve this, it is important to make all cuts as clean and straight as possible and to tie the graft union securely so that there is no movement in it.

Cleft grafting

Many techniques are used for grafting, and as long as safety is considered and a clean, straight cut is achieved, many of them are appropriate for coffee. Cleft grafting is our method of choice for grafting 'Guatemalan' coffee scions onto the nematode-resistant Fukunaga rootstocks. It was originally developed in Guatemala in the mid-1930s. Since that time, cleft grafting has been adapted and modified by several researchers. In Kona, Dr. Phil Ito first introduced this grafting method to evaluate several rootstocks for agronomic performance and for nematode control. The basic steps of the cleft grafting procedure are as follows:

- cut off the shoot tip of the rootstock with a sharp blade, leaving about 1–2 inches of stem
- make a V-shaped notch (cleft) in the rootstock
- remove the shoot tip from the scion
- trim the scion base to a V-shaped point matching the notch in the rootstock
- insert the scion into the cleft so the cambium layers are aligned
- wrap or use a clip to secure the scion/cleft union.

Note: the rootstock should be cut to produce a clean, smooth cut that is perpendicular to the long axis of the stem. Allow some space to open (allow the cleft to separate) while inserting the scion. It is very important to match the cambium of the scion with the cambium of the rootstock so that they come into direct contact. Secure the newly grafted plant with a clip, or wrap it to prevent drying and to prevent invasion of the wounds by pests or pathogens. Place the freshly grafted plants into a humid chamber until the union is healed and the plant is growing.

A checklist for cleft grafting coffee

Rootstock seeds should be scarified and germinated within a week of harvesting while they are still viable.

Start the rootstock in a sterile potting medium (or non-infested soil or other mix) to minimize the chance of any nematodes or other pathogens infecting the roots.

Germinate Fukunaga rootstock seeds 2–3 weeks before germinating 'Guatemalan' coffee seedlings to use as scions. Keep the seedlings moderately moist to prevent dehydration of tissue or rot by excess water.

The preferred scion material for grafting is a newly germinated seedling known as a "soldier." This stage is just before the first leaves appear.

The rootstock and the scion should be the same diameter to match the opposing cambium layers of the rootstock and the scion.

A sharp blade (a razor-sharp knife with a thin blade, or a razor blade) is required for making the cuts. A cleaning solution (ethanol, isopropyl alcohol, diluted bleach) should be at hand to clean the blade between cuts.

Ensure clean, smooth surfaces for all cuts to minimize rotting.

Place the grafted plantlet in a location that will minimize drying of the graft union.

After joining the cambium tissue, a grafting clip or plastic tape should be used to wrap the wound. It should be left on until the cut is completely healed, usually 3 weeks after grafting.

Grafted plants should be transplanted into a larger container that prevents drying out of the root system and that minimizes a "root-bound" (J-root) condition.

We recommend a seedling transplanting age of 12–15 months from grafting. Avoid planting them too young or too old.

Coffee seedling production for new plantings or for replanting nematode-infested coffee fields

Coffee nematode decline and the replant problem

Some coffee growers may find it necessary to replant portions of their farm due to coffee nematode decline. Other farmers want to start new farms. It is *essential* that only nematode-free coffee seedlings be used to start or replant a coffee farm in Kona. If young coffee seedlings become infected with the Kona coffee root-knot nematode (*Meloidogyne konaensis*) the plants may not survive more than a few years, or will certainly be less productive than healthy trees, may decline rapidly, and will be less tolerant of drought.

Tips for growing nematode-free coffee seedlings

Here are some suggestions and recommendations (some "DOs and DON'Ts") for the production of coffee seedlings in areas of Kona where management of coffee nematode decline is a primary concern.

DO

- Start and grow coffee seedlings in sterile media when possible, never in untreated field soil.
- Treat field soil with heat, steam, or another method of killing nematodes prior to use for coffee seedlings.
- Grow coffee seedlings in as large a container as is affordable (e.g., paper sleeves, plastic containers), and plant seedlings that are about 9–12 months old. Use resistant rootstocks if the nematode is present. Carefully avoid J-roots when transplanting.
- Grow seedlings on an elevated bench and over covered ground to avoid soilborne nematodes that may splash or be carried to seedlings.
- Try to isolate your nursery from your production fields and, if possible, place the nursery up-slope from nematode-infested fields and out of the path of water that may drain from nematode-infested fields.
- If possible, purchase coffee seedlings from a reputable nursery and bypass on-farm production of seedlings.
- Try to use coffee seedlings that are adapted (i.e., are grown) in your specific area, to reduce the incidence and severity of transplant shock.
- Inspect the nursery plants periodically for plant stunting, leaf yellowing, and for swellings and galls on roots.

DON'T

- Don't use *pulapula* seedlings; avoid their use at all costs, especially if they come from a nematode-infested farm.
- Don't use untreated field soil to fill bags or containers for growing coffee seedlings.
- Don't introduce nematodes into your nursery on your tools, equipment, boots, etc.
- When watering, don't splash water from ground to plants (or, try to minimize splashing).
- Don't plant coffee seedlings in nursery beds that contain untreated field soil.

Acknowledgment

Some of this information was derived from workshops conducted by Mario Serracin, CTAHR Department of Plant and Environmental Protection Sciences.