Testing the Hypothesis in Practice

If you want to test the theory in practice with one or more tree species, the first thing to do is to look for superior trees. This can mean many things: like trees that produce very large fruit, nut or pod crops; trees which produce larger than average nuts or fruits; trees which have smaller than average seeds; trees which produce sweet fruit instead of fruit too sour for human consumption; or trees which have a better than average ratio between the edible and non-edible portions of the fruit. If timber production is a factor, you might look for trees that have both large fruits and a long, straight stem.

Some of the desired characteristics may be exceedingly rare, and very difficult to find, especially if the tree species in question is rare or endangered. One German plant breeder once had to investigate 1.2 million plant samples before he found eight individuals that contained the desired gene producing sweet edible plants.

Some of the trees that are being domesticated by a non-governmental organization called the Veld Products Research and Development (VPRD) in Botswana are now increasingly rare. The Kalahari bushmen can walk for days to visit a certain individual wild orange or monkey orange tree growing in the wilderness. Because VPRD collects superior specimens from an area that is approximately 60 million hectares in size, it has naturally been impossible for it to do all the work by itself. VPRD has solved the problem by organizing competitions in schools and villages. The person who finds the tree that produces the largest or sweetest fruits, or the best fruit crop, wins a small prize.

This rather innovative approach has produced very good results. More than 10,000 people, mostly school children, have already participated in the search for superior specimens, and the competitions have produced a lot of valuable genetic material.

Without the participation of the 10,000 volunteers, it would have been virtually impossible for the VPRD staff to cover the vast land areas which have now effectively been combed by the children. In most cases the children had been herding cattle or goats in the bush, and already knew the superior trees from their previous experience.

Somewhat similar methods have been, and can still be, used in other countries as well. A small professional team of scientists coming from another part of the world and making a quick seed-collecting roundtrip can never find the best specimens in the vast areas of land that ought to be searched. However, the materials collected for tree-breeding work have often been acquired through such missions.

After finding the superior phenotypes, you have to find how you can propagate the superior trees vegetatively (how to make exact copies or clones of them). Sometimes this is very easy, because many trees can easily be grown from cuttings taken from stems, leaves, leaf-buds or roots. The procedures you should follow differ from one species to another, so you should check what is the right method from someone who knows, or proceed through experimentation (through the effort and failure or trial and error method), if the species is less well-known. In dry or cold areas, it is often best to take cuttings during the dry season or cold season, when the trees are resting and do not have any leaves. If the trees have leaves, it is often a good idea to remove them so that the cutting doesn't lose its moisture content through the leaves before it has grown its own roots. With some species, it is a good idea to put the cuttings into water and let them grow some roots in it before you plant them.

In commercial production, a plant hormone called auxin (the rooting hormone of the plant) is often used in order to hasten the process of root formation. Many species that would not otherwise grow well from cuttings can be propagated this way if auxin is used.

Some species produce a lot of root suckers (shoots coming from its root system). Such species are very easy to propagate vegetatively: you only have to separate a root sucker and some of the adjoining root system from the mother tree and plant it in another place. Some species can be propagated from various specialized organs like bulbs, corms, rhizomes, tubers or tuberous roots that the plant is using for its own food storage.

However, many species do not produce root suckers, do not have suitable food storage organs and do not grow well—or at all—from cuttings. Such species are more difficult to propagate vegetatively, and require more complex techniques.

One group of vegetative propagation techniques involves the joining of two plant parts together so that they grow like one plant. The part consisting of the root system and a stem is called the stock plant (or rootstock). In grafting, a whole cutting is set into the rootstock, in budding, only a bud is used. The common name for buds and grafts is scion (*see drawings of processes on following pages*).

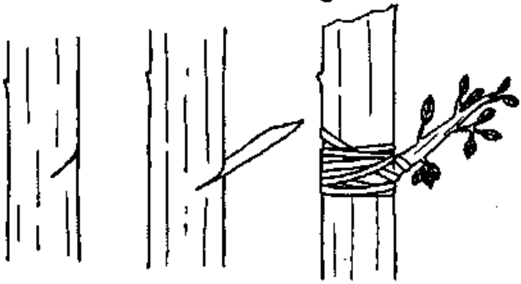
In grafting and budding, the rootstock and the graft (or the bud) are placed so that their tissues will remain in close contact and grow together before the surfaces dry. Large numbers of vegetatively propagated seedlings can be produced by these methods, which are especially useful in the propagation of species that are difficult to root. There are numerous different grafting and budding methods, but we will not go into the details here.

Another set of methods is called layering. Layering basically means that a shoot of a plant is encouraged to start producing roots (so-called adventitious roots) while it is still attached to the mother plant. When these adventitious roots are well developed, the shoot can be cut loose and separated. Layering is easier than grafting or budding, both of which require some practice and skill. However, layering is a very labour-intensive method, and only relatively small numbers of new plants can be produced. Also, the new plants propagated by layering tend to have shallow root systems. However, if we only want to establish a seed orchard, we do not have to worry about this.

In simple layering, a low, flexible shoot which bends freely is bent slowly to the ground and then buried in soil, five to seven centimetres deep. The tip of the shoot is left exposed, and the side shoots 15 or 20 centimetres from the tip are removed before the shoot is buried. The soil surrounding the buried shoots should be kept moist. In tropical conditions the rooting usually takes from five to seven weeks, in temperate zones, even two years are sometimes required. Some species root faster than others.

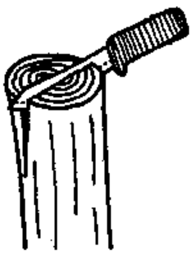
In trench layering, an entire branch or plant (sometimes even a whole tree) is bent slowly into a shallow trench and covered with five to seven centimetres of soil. As the shoots emerge, more soil is put on top of them so that they do not get any light, and start to

Side Grafting



A slanting cut is made in the root stock (the tree with the roots). A section is inserted in the cut. The stock is cut back when the scion has started to grow.

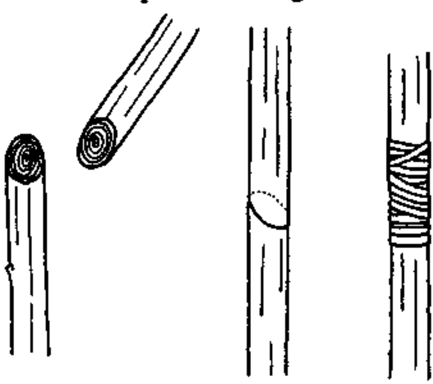
Cleft Grafting



Vertical split is made on stock

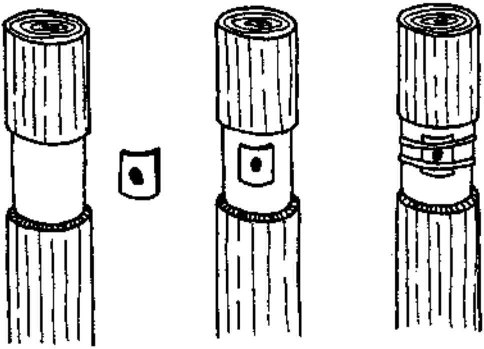
The split is held open with a wedge, the prepared scion is inserted and the wedge is withdrawn.

Splice Grafting

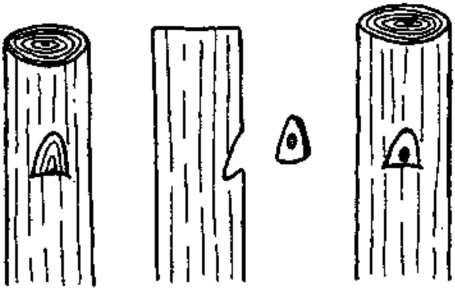


The stock and the scion are cut so that their surfaces match perfectly, then placed together and tied together with tape.

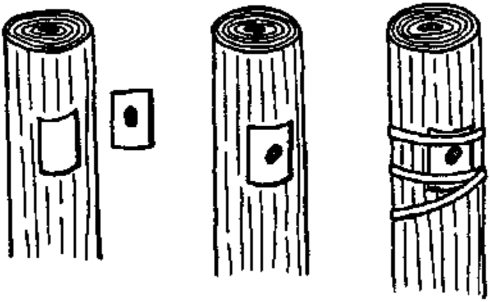
Ring Budding



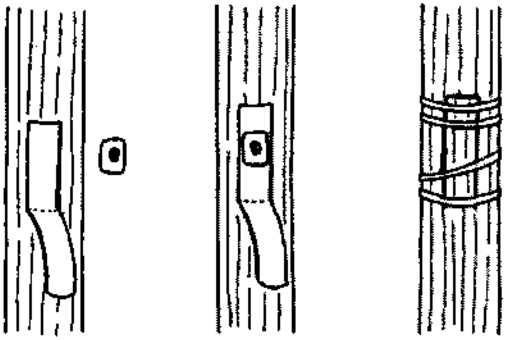
Chip Budding



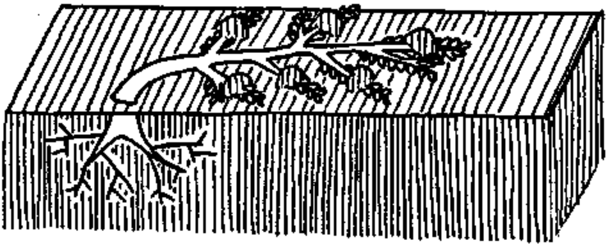
Patch Budding



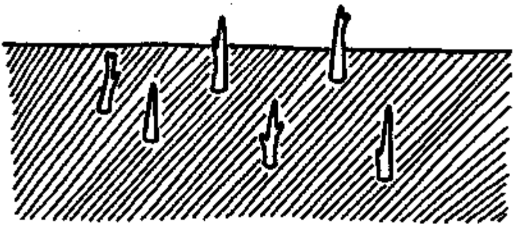
Budding by the Folkert method



Trench layering

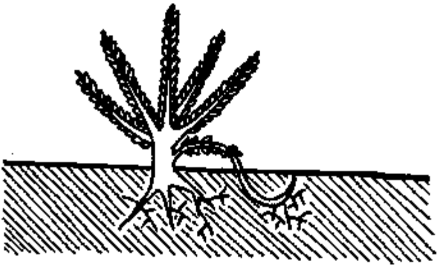


In trench layering the whole mother plant is bent down
on the bottom of the trench, pegged down
and covered with soil.



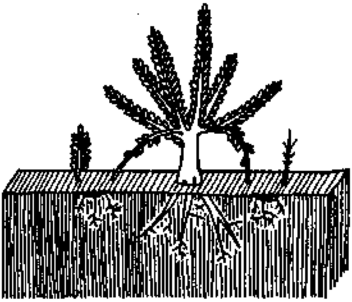
When new shoots emerge, soil is added to cover their bases.
Finally the soil is removed and the rooted shoots
are separated from mother plants

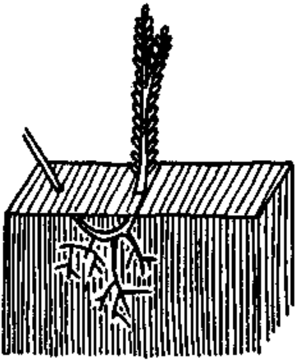
Tip Layering



In tip layering the current season's shoots are used.

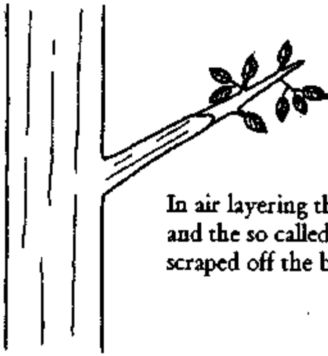
Simple layering



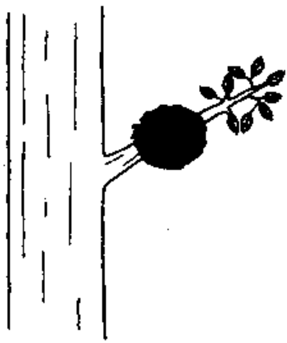


After the buried part has grown its own roots
it can be transplanted.

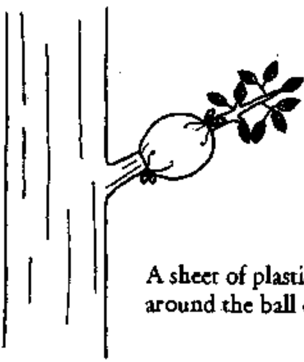
Air layering



In air layering the bark is removed and the so called cambium layer scraped off the branch.

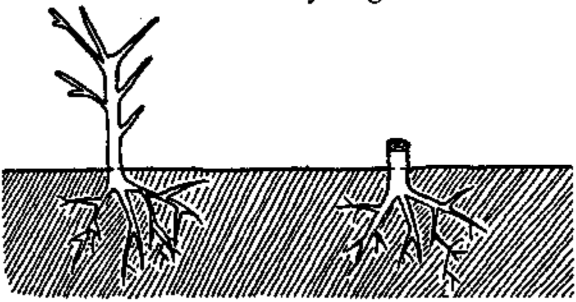


A ball of moist sphagnum moss or soil is placed around the ring-barked section.



A sheet of plastic is wrapped around the ball of moss (or soil).

Mound layering



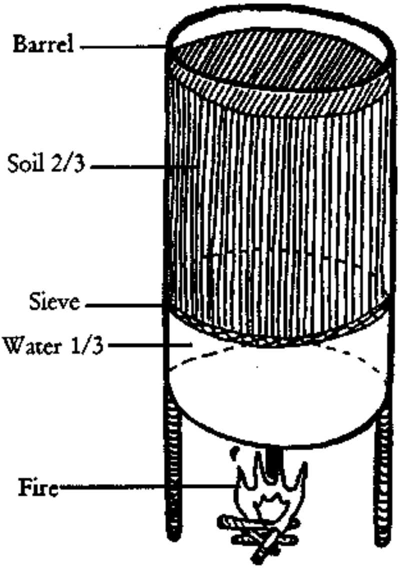
1. 2.

In mound layering the top of the mother plant is removed and the stump is covered with soil.

3. 4.

5.

If the mother plant / tree is small and bushy it can be buried as a whole, without removing the top.



The soil sterilisation method used by VPRD and CED in mycorrhiza studies. Water heated by the fire becomes steam that kills all mycorrhiza spores in the soil. After the soil has cooled, the spores of the desired mycorrhizas can be introduced.

produce roots. When rooting is complete, the soil is removed and the rooted shoots can be separated from the mother plant and transplanted.

In air layering, fairly long, partially hardened, one-or-two-year-old shoots are used. All the leaves of the shoot, except the first few ones near the tip, are removed. The shoot is ring-barked: all the bark is removed from a length of 1.5 to 2.5 centimetres. Moss or moist soil is placed around the ring-barked section of the shoot, and a transparent plastic sheet is wrapped around it. The ends of the sheet are twisted and tied tightly. The plastic prevents the moisture from escaping, and roots should develop within 30 to 45 days in tropical or subtropical conditions. In India, the ideal time for air layering is early spring, or the onset of the monsoon.

In mound layering, the stem is cut back to a height of 10 centimetres above the ground level (during the dormant season). When the shoots emerge, they are buried. When they emerge again, more soil is added on top of them. The soil is kept moist in order to encourage the rooting.

The first aim should always be to grow a small plantation of trees, each of which is—genetically speaking—an exact copy of a superior tree found somewhere in the wild, or in a farmer's backyard. When the trees grow and start to produce fruit, we may find that some of the superior trees were only superior phenotypes and not superior genotypes. The quantity and quality of fruit produced by a tree is influenced by a combination of genetic and environmental factors. Even if a tree is producing a good crop, this doesn't necessarily mean that it has superior genes. It may be that the large fruit crop is mostly caused by environmental factors. It may, for instance, be that the tree is growing at a very favourable site. The site may have been a large termite mound, which means that there is a lot of natural fertilizer in the soil. Or it may be that there is an abundant underground source of water.

The characteristics visible in a tree, caused by a combination of environmental and genetic factors, form a phenotype. A genotype refers to the characteristics that are of a genetic origin, and not caused by environmental conditions.

When the vegetatively propagated clones of various superior trees are planted in a garden, the unfair advantages from environmental factors are eliminated. This means that the trees whose fruit crops were based on favourable environmental conditions do not perform very well. But the trees whose desired characteristics were based on genetic factors should produce good crops even in the new location. We should keep such trees and remove the rest.

In an official, scientific plant-breeding station, pollination (fertilization) is done by technicians with tweezers, so that it is known exactly which tree has fertilized a certain flower. Scientists keep precise records of the lineage of each individual. If we really want to wipe out the genes producing undesired characteristics, we should follow this procedure. It is the only way to find out which of the parent trees contain and which do not contain recessive genes causing the undesired characteristics. We shall come back to this a little later.

However, if our aim is to produce genetically diverse stands of trees, we do not necessarily have to be quite as precise as this. If all the trees growing in our seed orchards have the desired characteristics, we can let them fertilize each other randomly, just like it would happen in nature. If all the trees are carefully selected superior genotypes, and if there are no inferior trees of the same species growing nearby, the seed produced by our trees should mostly produce good-quality offspring.

As mentioned before, the desired characteristic (caused by only one gene) visible in a tree can be caused either by two recessive or two dominant genes in both parents; by two dominant genes in one and one dominant gene in the other parent or by one dominant gene in each parent. Only in the last option is there a chance that some (25 per cent) of the offspring will not contain the desired characteristic.

Let's say that we are interested in three characteristics, which are: large fruit crop, large size of the fruit and the sweetness of the fruit. We have selected only trees that contain all the desired characteristics. Let's assume that the large fruit crop is not, in reality, an important quality, because all the wild trees produce heavy fruit crops. So, in practice, we can concentrate on two qualities. We will also assume, in this example, that none of the important characteristics are inherited together, and that they can thus be separated by selective breeding.

To be on the safe side, we can start with the worst option, and assume that both parent trees have a recessive gene that causes sour fruit, which means that 25 per cent of the first-generation offspring will produce sour fruit. (If only one or neither parent had such a gene, none of the trees would produce sour fruit.) Let us also assume that both parents have a recessive gene related to small fruit size. Because both parents have both undesirable, recessive genes, only nine out of 16 of the trees growing from their seed will produce large and sweet fruit. Three produce large but sour, three sweet but small and one small and sour fruit.

If our gardener is only interested in large and sweet fruit, he has grown seven useless trees for each nine useful trees. However, there are at least two easy solutions to this problem. One is to begin with more trees that can finally remain on the plot. This can easily be done, because small trees require much less space than mature ones. Also, the price of seed-grown trees is much less than that of grafted seedlings. If you establish the trees from seed yourself, you only need to purchase or acquire the seed.

This way you can start, for example, with 1,600 trees per hectare and remove up to 700 of them, when you can see which ones do not contain all the desired characteristics. Such a method, however, may contain some opportunity costs, because it limits the possibilities for growing other crops between the trees while they are still small.

Another possibility is to establish the actual seed lots only after identifying and eliminating the trees that contain undesired recessive genes. Official plant breeders wrap some of the female flowers of the superior trees with plastic or with pieces of canvas, in order to prevent random pollination by insects (or by wind, if the species is pollinated by it). Then some pollen is taken from the male flowers of another tree and put into the protected female flowers. Other female flowers of the same tree are fertilized with pollen taken from the male flowers of another tree, and so on. Everything is recorded, meticulously and precisely.

We now know both the parents of the trees grown from the seed produced by the artificially fertilized flowers. The characteristics of the first-generation offspring already tell us a lot. If all the trees produce only the qualities we have been hoping for, we know that their parent trees (or clones of them) can only produce first-generation offspring that always have the desired characteristics. This means that the parent trees (or clones of them) can be used as mother trees in seed lots with very good results. We cannot yet promise that this would apply to their grandchildren as well, because one of the parents might still have an undesired recessive gene.

However, if we have done a number of experiments with different trees, the results of the other trials might tell us whether this is true or not. To make sure, we can cross some of the first-generation offspring with each other. If the undesired characteristic doesn't appear in the second generation, we can safely assume that our seed is a stable variety and breeds true from generation to generation—in terms of certain characteristics. If we want to breed more stable characteristics into our seed, we need more generations to do this—and much more space for the trials. But as long as we are only dealing with a few qualities, the effort is manageable even on a small farm.