# Foundation Clonal Systems of Source Selection

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# INTRODUCTION

Plant breeding and plant propagation can be considered mirror images of one another. Plant breeding depends upon increased genetic variation to provide the chances of producing new cultivars. Plant propagation, on the other hand, depends upon decreasing, or at least controlling, genetic variation in order to maintain the genotype of specific cultivars produced by breeding. Maintaining plants that are true-to-variety and true-to-type is accomplished through selection of propagation sources. One of the primary methods of accomplishing this goal is through vegetative propagation and the selection of a clone. A further refinement of clonal selection and propagation is the selection of individual plants within that clone and will be referred to as foundation clones.

The purpose of this workshop is to describe the types of clonal variants that need to be controlled and the process utilized to achieve this control through "Foundation Clone" selection. These procedures are embodied in the California Registration and Certification programs.

# KINDS OF VARIATION

Propagation sources in horticulture are either seedling or clonal. Seedling populations exhibit both genetic variation and nongenetic variation. Seedling sources are populations of individual plants, each of which have different genotypes.

**Genetic Variation.** This type occurs among individual seedlings resulting from segregation of chromosomal DNA during sexual reproduction. DNA controls the genetic codes that determine plant characteristics. Variation among individual seedlings within a population is controlled by managing gene frequencies through plant breeding techniques.

**Nongenetic Variation.** This type develops within individual plants as the seedling grows and completes its life cycle. The process is controlled by the transcription and translation of the specific genetic codes in the DNA in relation to age, season, and pattern of growth. This type of variation is defined by biologists as *epigenetic* and in horticulture includes the phenomenon known as phase change which utilizes the terms juvenile, transitional, and mature (adult) to different phases in the life cycle. Variations can be expressed in: (a) the age of flowering, (b), growth and foliage characteristics, and (c) the ability to regenerate adventitious roots.

Vegetative propagation can be used to control both genetic and non-genetic variation within seedling plants. The process of vegetative propagation, also referred to as *cloning*, creates a unique kind of plant population which is known biologically as a *clone*, all plants of which have the same genotype.

**Origin of Clones.** Most horticulturally important clones originate by the selection from a seedling population of a single individual. This process can result in a very large genetic advance in one step and the resulting genotype can theoretically be

maintained indefinitely through clonal propagation. In practical horticulture, this statement is not historically true. As horticultural industries have evolved and expanded, specific kinds of clonal variation, including both disease and genetic modifications, have also emerged that may create serious problems of trueness-to-type, involving plant modification or change in performance. The need to control such problems has led to revolutionary changes in the traditional methods of source selection.

Variation patterns within clones can be conveniently designated by the consecutive generation's vertical propagation. The original seedling plant is designated as  $S_0$ , the first scion generation as  $S_1$ , the second scion generation as  $S_2$ , etc. Horizontal propagation is multiple propagations from the same plant or from the same vertical generation. Plants of the same horizontal generation can be designated by lower case letters, as  $S_{1a}$ ,  $S_{1b}$ ,  $S_{1c}$ , etc.

### PHASE CHANGE AND VEGETATIVE PROPAGATION

Although usually restricted to the first few generations of vertical propagation,  $S_0$ ,  $S_1$  and sometimes later, juvenility and maturation provide important examples of non-genetic variation which can have profound effects on trueness-to-type of many plants. As seedlings, many perennial plant species, i.e., trees, bulbs, tubers, orchids, etc., require many years to come into flowering, show striking morphological differences between the juvenile and mature parts of the same plant and lose their ability to reproduce vegetatively as they age. Vegetative propagation of buds from different parts of the  $S_0$  plant may result in horizontal variation among  $S_1$  plants depending upon the location of the meristem on the original seedling plant source.

Proper selection and maintenance of source material from juvenile parts of the clone are necessary to maintain ease of rooting and specific growth forms in clonal forestry, for instance, or for growing clonal rootstocks.

Most clonal cultivars grown for their fruits and flowers, however, become stabilized to the mature (adult) phase with repeated vertical and horizontal propagation. These characteristics include the more desirable horticultural characteristics (precocious bearing, somewhat less vigor, good fruit quality, thornlessness) whereas the juvenile form is often highly vigorous, thorny, produces poor quality fruit, and has a tendency toward upright growth. Loss of rooting ability requires that many of these clones be propagated by budding and grafting.

# PATHOGENS, INFECTION, AND DISEASE WITHIN CLONES

The most serious problem of clonal variation in long-term established cultivars after repeated horizontal and vertical propagation is systemic infection by pathogens. Historically, many if not most clones eventually show declines in yield or may actually die out, a phenomenon referred to in fruit growing as "running out". The cause was attributed initially to old age of the clone which had to be rejuvenated by creating a new cultivar by seed propagation.

Eventually scientists discovered that most of these problems were caused by pathogens. Bacteria, fungi, and other organisms were identified which adversely affected both seedlings and vegetatively propagated plants. This led to the concept of pathogen-free stock controlled through a variety of sanitation, heat treatments, and chemical techniques. However, other plants, primarily woody plants, and including most fruit and nut trees, small fruits, strawberries, grapes, potatoes, and

other clonally propagated cultivars, became almost systematically infected with submicroscopic organisms as viruses, viroids, phytoplasma, etc. These pathogens literally became part of the genetic system of the host in some cases. These were propagated along with the cultivar, transmitted across graft unions and transmitted by insect vectors (particularly aphids) and pollen. By the mid-1950s certain fruit industries in California, for example cherry in coastal valleys and peaches in southern California, were being decimated by serious viruses. Subsequently, various fruit and nut crops became affected by a series of virus-induced disasters, including citrus decline, walnut blackline, and pear decline.

#### FOUNDATION CLONE SELECTION

Research by plant pathologists discovered indexing methods of detection for most viruses through transmission to susceptible indicator plants. These tools led directly to the method of control which is called here Foundation Clone Selection based on the identification of single plants which tested free of significant pathogens. Reinfection was prevented by growing the Foundation Trees in a Foundation Orchard followed by multiplication (horizontal propagation) in nursery source orchards (mother blocks, scion orchards, or nursery increase blocks) for limited numbers of vertical propagation generations. These procedures were embodied in the California Registration and Certification system based upon the well established Registration and Seed Certification System. The source plant and its progeny were referred to as a clone which is biologically correct, but fails to distinguish this category of plant from the original clone of which it was a part. It is not a new cultivar. For this reason, it would be better to refer to it by another term as Foundation Clone although other terms have been used, including source-clone and nuclear stock.

Three methods can be used to identify foundation clones. One is already described and involves screening of available plants. A second method utilizes plants in which the virus has been eliminated from infected plants, primarily through thermotherapy and meristem culture. This advance makes possible the selection of genetically superior material as the primary step and the removal of the virus as the second. A third method is used in citrus with the selection of virus-free apomictic seedlings of established cultivars followed by vertical propagation to establish the horticulturally desirable mature form.

# **GENETIC NATURE OF FOUNDATION CLONE SELECTION**

Events occurred in the early stages of program development that reinforced the concept that Foundation Clone selection is not just for disease control, but is also a genetic process. A single plant is chosen within the entire clone to represent the entire cultivar. It is essential that this plant be verified as true to variety. As it happened, a Foundation Clone of a red-fruited cherry cultivar selected for the program turned out to produce white fruit, evidently due to an error in selection at some stage. Although momentarily embarrassing, the event emphasized the importance of this verification step. This event led to the practical rule to collect propagation material only from a plant whose genetic identity had been verified at the fruiting stage by visual inspection.

Trueness-to-Type. This term is sometimes used synonymously with trueness-to-variety. A distinction should be made in that the plants to be used as a propagation

source may actually be the correct cultivar but deviate in some fundamental way from the standard for that cultivar. When propagated, off-type individual progeny result, a fact not necessarily predicted from visual inspection of the source plant. This distinction brings up an important concept:

Phenotypic Selection. Selection based on visual inspection of the source plant.

**Genotypic Selection.** Selection based upon visual inspection of the progeny plants. The procedure requires propagations be made and the progeny plants grown in appropriate test sites. Plant breeders use this procedure to evaluate a new cultivar but plant propagators often may not utilize it in evaluating sources.

# **NONPATHOGEN VARIANTS IN CLONES**

Mutations are produced by genetic changes in the basic structure of DNA which results in a new genotype. These events occur at random within clones and can be an important source of new cultivars. Equally probable is that mutations can produce permanent changes that are adverse (reduced yield, reduced quality, inferior growth). These changes are not necessarily identified in the source plant, but may only appear later in the progeny plants (often in the customers orchard).

New mutations occur in single cells which are located in specific layers of the growing point and invariably become unstable chimeras. Genotypic selection can identify unstable plants because new plants arise invariably from single buds.

Mutation breeders generally utilize one or more generations of vertical and horizontal propagation to identify new mutants. A late-blooming budsport was discovered in almond in the early 1950s, not in one tree but as single limbs or whole trees in several separate trees, all apparently arising as nursery trees from single buds taken from a single tree where the mutation had occurred. It was a single factor mutation. Later on, a type of variant began to appear in commercial orchards, characterized by low production, vigorous growth, abnormal fruit, and extreme variability within and among tree. It appeared to be a multiple factor mutation which was eventually traced through two major nursery propagation source lines, apparently arising from some event in the environment some years previously. Foundation Clones can control these problems providing that the Foundation Clones were previously subjected to genotypic selection.

Hereditary Disorders. Noninfectious bud failure (BF) is a unique disorder that develops in specific propagation lines of particular almond cultivars. It produces a "witches-broom" type of phenotype (also known as "crazy-top"). Vegetative buds are killed in late summer and fall, resulting in bud-failure, dieback, and vigorous growth from surviving buds in the spring. The disorder is not pathologic, but the potential for BF is inherited during breeding and develops over time in clonal source material of individual cultivars. Enhancement of the potential increases during vegetative growth, the rate directly proportional to accumulated exposure to temperatures over 32C (80F). In the traditional nursery propagation orchard selection systems used in the past, the potential for BF increases during consecutive vertical propagations and variation increases with horizontal propagation. Control has been achieved through pinpointing specific Foundation Clones whose potential for BF has been previously progeny tested by genotypic selection.

Genotype × Environmental Interactions. This phrase means that the expression of specific genotypes sometimes results from interactions among genotype, environment, and (or) management. Visual inspection of source plants for trueness-to-type can sometimes be misleading since a specific variant may be due to an environmental interaction. Commercial fruit quality of such plants as apples, peaches, plums etc. depend upon restricting the crop load on the tree by thinning and maintaining vigor and other characteristics. Time of bloom may depend upon the amount of chilling in the winter as well as warm temperature patterns in spring. Time to harvest may depend upon the temperature accumulation during development or the amount irrigation, etc.

When we were studying the so-called "bull" problem in almond, observations were made of Foundation Clone trees in scion orchards which had been severely pruned for budwood, and showed bull characteristics. Questions were raised if these specific Foundation trees were off-type. Accompanying studies showed that these plants were not off-type and the symptoms were due to management conditions. Similarly, young nonbearing trees in orchards may show off-type fruit symptoms. This phenomenon can be a problem in Foundation Clone selection and management because maintenance of trees for production of propagating material is radically different than optimum for fruit or nut production. This potential problem further emphasizes the importance of genotypic selection in the kind of Source Selection system described here.

# **SUMMARY**

Clonal propagation through vegetative propagation is the dominant method of producing woody and herbaceous perennials in horticulture. Although a powerful tool for selecting superior cultivars and maintaining their genotype in propagation, their use requires special methods of source selection to maintain their trueness to type and freedom from disease. It is evident that coevolution of pathological organisms and clonal cultivars has been a fact of horticultural development and specific programs of source selection has become a fact of modern propagation systems. The principles of Foundation Clone selection and maintenance have become a necessity. This workshop described the specific application to orchard and vine crops in California.

# LITERATURE CITED

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