APPLES

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The apple is the most ubiquitous of temperate fruits and has been cultivated in Europe and Asia from antiquity. It was known to the Greeks and Romans and mentioned by Theophrastus in the third century B.C. Since then the apple has been distributed into almost all parts of the world. The genetic variability found in the apple has allowed adapted types to be selected for different environments, and selection continues for new types to extend apple culture into both colder and warmer regions. Orchards are now found in Siberia and northern China where winter temperatures fall to –40°C and in high elevations in Colombia and Indonesia straddling the equator where two crops can be produced in a single year (Janick 1974). Present world production of apples (FAO 1995) is close to 49 million tonnes. Apples are the fourth fruit crop in importance after all citrus (85 million t), grapes (56 million t), and banana (53 million t). The leading countries for apple production are presented by continent in Table 1.

Apples are popular because of the many ways that they can be consumed and because of their convenience and durability. Apples may be eaten off the tree or stored for up to a full year. Apples can be processed into sauce, slices, or juice and are favored for pastries, cakes, tarts, and pies (Downing 1989). The pulp has been processed into candies (fruit leathers) and used as a source of pectin. The juice can be consumed fresh, either natural or filtered, fermented into alcoholic beverages such as cider or wine, distilled into brandy, or transformed into vinegar. Apples have become the symbol of wholesomeness: "An apple a day keeps the doctor away" is a favorite aphorism, and apple pie has become a symbol of goodness along with motherhood. Finally, crabapples, grown for their attractive flowers, foliage, and fruit, are among the most popular of ornamental trees.

At one time each country and area had its own local cultivars. This is still the case in some areas, but with expanding production and transport networks, and the

TABLE 1. World Production of Apples, 1994.

Principal apple producing country	Production (million tonnes)	Principal apple producing country	Production (million tonnes)
Asia	**20.4**	**North America**	**6.0**
China	12.0	United States	4.9
Iran	1.6	Mexico	0.5
India	1.2	Canada	0.5
Japan	1.0		
		South America	**2.7**
Europe	**13.2**	Argentina	1.0
France	2.2	Brazil	0.8
Italy	2.1		
Russian Federation	1.7	**Africa**	**1.3**
Romania	1.4	South Africa	0.6
Poland	1.4	Egypt	0.3
Germany	0.9	Morocco	0.3
Spain	0.7		
Hungary	0.7	**Oceania**	**0.9**
Netherlands	0.7	New Zealand	0.5
		Australia	0.3

Source: FAO (1995).

requirements for high yield of commercial quality, a few cultivars dominate all the major apple growing areas. For example, the most widely grown cultivars by far are 'Golden Delicious' and 'Delicious' and its red sports, both chance seedlings of American origin. 'Golden Delicious' has been widely and successfully used in breeding, and its seedlings, which make up a high proportion of the new cultivars, are rapidly changing the apple industry. Since the apple is a long-lived tree and vegetatively propagated, cultivars known hundreds of years ago still exist. Apples that date to antiquity have been collected in Italy (S. Sansavini, personal communication). The large world collections and germplasm repositories are living museums that show the development and improvement of the apple and contain a vast reservoir of desirable genes. Old cultivars are little used by breeders because their seedlings generally produce fruit inferior to that of the cultivars being grown today, but they represent a living gene bank. Most breeding in recent years has been among the best commercial cultivars. Interspecific hybridization to introduce new genes to scion cultivars as well as rootstocks is being used, but this requires a lengthy breeding process. To overcome these limitations of time and space, recent advances in biotechnology involving gene transformation now make it possible to introduce genes from almost any source into apple.

ORIGIN AND EARLY DEVELOPMENT

The apple, in company with most of the important temperate fruits-pear, plum peach, apricot, cherry strawberry, raspberry, and blackberry-belongs to the Rosaceae or

rose family. Apple, pear, quince, medlar, and some less well-known genera have been classified into the subfamily pomoideae, the pome fruits. These are characterized by fruits consisting of two to five carpels enclosed in a fleshy covering. The genus *Malus* has, according to most authorities, 25 to 30 species and several subspecies of so-called crabapples, many of which are cultivated as ornamental trees for their profuse blossom and attractive fruits (Table 2). Most of the species intercross and, since self-incompatibility is common, trees produced from seed obtained from a botanic garden or arboretum where collections of *Malus* are grown are almost always interspecific or intercultivar hybrids. it is therefore very difficult to be certain of the authenticity of specific names.

The cultivated apple is likely the result of interspecific hybridization and at present the binomial *Malus ×domestica* has been generally accepted as the appropriate scientific name (Korban and Skirvin 1984). The main ancestor of apple is now considered to be *Malus sieversii,* which is wild from the Heavenly Mountains (Tien Shan) at the boundary between western China and the former Soviet Union, to the edge of the Caspian Sea (Morgan and Richards 1993). This species is diverse and wild trees bearing the full range of forms, colors, and tastes are found in Kazakhstan and other independent countries of Central Asia formed from the breakup of the Soviet Union and especially around Alma Ata (Father of Apples). This is the area of greatest diversity and the center of origin. Recent collection trips to central Asia have verified that *M. siever3ii* is very diverse and has all the qualities present in *M. ×domestica* (Forsline et al. 1994; Forsline 1995). Vavilov (1930) in his explorations found Trony wild apples in woods in the Caucasus and Turkestan bearing firuits within a wide range of sizes, some of quite good quality. Species that have contributed to the genetic makeup of the apple include *M. orientalis,* which bears late-keeping bitter fruit; *M. sylvestris,* the European crab, bearing small astringent, greenish-yellow fruits, native to an area that extends from Britain across Europe to the Ball-an and northern Turkey; and a number of species from eastern Asia, including *M. baccata,* the hardy but small Siberian crab, *M. mandshurica,* the Manchurian crab; and *M. prunifolia*, the larger Chinese crab. A list of *Malus* sections, primary species, and species hybrids is presented in Table 2.

Cultivation of the apple seems to have been practiced by the Greeks and Romans and, as a result of their travels and invasions, to have been spread by them throughout Europe and Asia. Later cultivation was concentrated around the medieval monasteries. Cultivars were selected and propagated in very early times, for grafting was known at least 2000 years ago. By the end of the thirteenth century, many named cultivars were known, and from this time we get the names 'Pearmain' and 'Costard'. The history and romance of the apple has recently been summarized in *The Book of Apples* by Morgan and Richards (1993).

Until the latter half of the twentieth century most of the world's apple cultivars were chance seedlings selected by fruit growers. More than 10,000 cultivars are documented, yet only a few dozen are grown on a commercial scale worldwide (Way et al. 1990). In 1983, the best known cultivars in the world were all chance seedlings found in the eighteenth or nineteenth centuries, of which many were derived in North America: 'Golden Delicious' (6.3 million t, origin US), 'Delicious' (3.0

TABLE 2. *Malus* **Sections and Primary Species**

Sections	2n	Apomixis	Fruit size (cm diam)	Calyx[a]	Carpel no.	Persistance of ripe fruit
SECTION 1. Malus						
Subsection A. Pumilae						
Series a. Pumilae						
M. asiatica Nakai	34	No	>2	P	5	No
M. domestica Borkh.	34,51,68	No	>2	P	5	No
M. micromalus Makino	34	No	1	D	—	—
M. orientalis Uglitzk. ex Juz.	—	—	—	—	—	—
M. prunifolia (Willd.) Borkh.	34	No	2	P	5	Yes
M. pumila Miller	34	No	>2	P	5	No
M. sieversii (Lodeb.) M. Roemer	—	—	>2	P	5	No
M. spectabilis (Aiton) Borkh.	34,68	No	2	P	5	Yes
M. sylvestris Miller	34	No	>2	P	5	No
Series b. Baccatae						
M. baccata (L.) Borkh.	34,68	No	1	D	4, 5	Yes
M. floribunda (Siebold) ex. Van Houte	34	No	1	D	4, 5	Yes
M. halliana Koehne	34	No	1	D	3, 4, 5	Yes
M. hupehensis (Pampan.) Rehder	51	Yes	1	D	3, 4	Yes
M. mandshurica (Maxim.) V. Komarov	34	No	1	D	5	Yes
M. sikkimensis (Wenzig) Koehne ex C. Schneider	51	Yes	1	D	4, 5	Yes
Subsection B. Sieboldianae						
M. sargentii Rehder	68	Yes	1	D	3, 4, 5	Yes
M. sieboldii (Regel) Rehder	34-85?	?	1	D	3, 4, 5	Yes
Subsection C. Kansuenses						
Series a. Kansuenses						
M. fusca (Raf.) C. Schneider	34	No	1	D	2, 3, 4	Yes
M. kansuensis (Batalin) C. Schneider	—	No	1	D	3, 4, 5	Yes
M. komarovii (Sarg.) Rehder	—	—	1	D	—	Yes
M. toringoides (Rehder) Hughes	51	Yes	1	D	4, 5	Yes
M. transitoria (Batalin) C. Schneider	—	—	1	D	5	Yes
Series b. Yunnanenses						
M. honanensis Rehder	—	—	1.5	P	4	Yes
M. ombrophila Hand.-Mazz.	—	—	>2	P	5	No?

TABLE 2. (Continued).

Sections	2n	Apomixis	Fruit size (cm diam)	Calyx[a]	Carpel no.	Persistance of ripe fruit
M. prattii (Hemsley) C. Schneider	34	No	1.5	P	5	Yes
M. yunnanensis (Franchet) C. Schneider	34	No	1.5	P	5	Yes
SECTION II. Sorbomalus						
M. florentina (Zuccagni) C. Schneider	34	No	1	P, D	3, 4, 5	Yes
SECTION III. Eriolobus						
M. trilobata (Poiret) C. Schneider	—	No	2	P	5	—
SECTION IV. Chloromeles						
M. angustifolia (Aiton) Michaux	34	No	>2	P	5	No
M. coronaria (L.) Miller	51(68)	Yes?	>2	P	5	No
M. ioensis (Alph. Wood) Britton	34	No	>2	P	5	No
SECTION V. Docyniopsis						
M. doumeri (Bois) A. Chev.	—	No	>2	P	5	No
M. melliana (Hand.-Mazz.) Rehder	—	—	>2	P	5	No
M. tschonoskii (Maxim.) C. Schneider	34	No	>2	P	5	No

Note. Malus species hybrids include
M. ×adstringens Zabel. (*baccata × pumila*
M. ×arnoldiana (Rehd.) Sarg. (*baccata × floribunda*)
M. ×astracanica Dum.-Cours. (*pumila × prunifolia*)
M. ×atrosanguinea (Spaeth) Schneid. (*halliana × sieboldia*)
M. ×dawsonia Rehd. (*fusca × pumila*)
M. ×gloriosa Lem. (*pumila niedzwetskyana × scheideckeri*)
M. ×hartwiggii Koehne (*halliana × baccata*)
M. ×heterophylla Spach (*coronaria × pumila*)
M. ×magdeburgensis Schoch. (*spectabilis × pumila*)
M. ×platycarpa Rehd. (*coronaria × domestica*)
M. ×purpurea (Barbier) Rehd. (*niedzwetzkyana × atrosanguinea*)
M. ×robusta (Carr.) Rehd. (*baccata × prunifoia*)
M. ×scheideckeri (Spaeth)Zab. (*floribunda × prunifolia*)
M. ×soulardii (Bailey) Brit. (*ioensis × pumila*)
M. ×sublobata (Dipp.) Rehd. (*prunifolia × sieboldiii*)
M. ×zumi (Mats.) Rehd. (*mandschurica × sieboldii*)
[a]P, persistent; D, deciduous.
Source: Adapted from Way et al. (1990).

million t, origin US) 'Cox's Orange Pippin' (1.7 million t, origin England), 'Rome Beauty' (0.8 million t; origin US), 'Belle de Boskoop' (0.7 million t, origin The Netherlands), 'Granny Smith' (0.6 million t, origin Australia), 'Jonathan' (0.5 million t, origin US), 'Reinette du Canada' (0.4 million t, origin France), 'McIntosh' (0.3 million t, origin Canada), and 'Worcester Pearmain' (0.3 million t, origin England). The introduction of apples into the United States as seeds and the replanting of seeds obtained from cider mills resulted in literally tens of millions of seedlings being grown and evaluated by fruit growers. The nurseryman Jonathan Chapman of Leominster, Massachusetts, known as the legendary Johnny Appleseed, is credited with distributing apple seeds hum the cider mills of Western Pennsylvania throughout the wilderness of Pennsylvania, Ohio, and Illinois in the eighteenth century (Morgan and Richards 1993). The evaluation of these seedlings was the major achievement of United States pomology in the nineteenth and early twentieth centuries. The 'Delicious' apple (discovered in Iowa, 1872) still dominates United States production, and 'Golden Delicious' (found in West Virginia, 1905) dominates Europe. Other cultivars are important regionally such as 'McIntosh' in the northeastern United States and eastern Canada, 'Jonathan' in the Midwest, 'York Imperial' in the Shenandoah Valley, 'Granny Smith' in Australia, 'Cox's Orange Pippin' in England, and 'Belle de Boskoop' in The Netherlands.

The origin of controlled breeding of apples is attributed to Thomas Andrew Knight (1759-1838) who produced the first cultivars of known parentage. This technique continues to be the basis of all present day apple breeding programs but the process until recently had been notorious unsuccessful as compared to other fruits. In retrospect, the reason appears to be the poor choice of parents. In the first half of the twentieth century the only important US cultivars derived from controlled breeding were 'Cortland' ('McIntosh' × 'Ben Davis') and 'Idared' ('Jonathan' × 'Wagener'), despite the fact that hundreds of apple seedlings were named. However, in the last 25 years, seedlings derived from controlled hybridization began to find a place in world apple production. These include 'Elstar', 'Gala', 'Jonagold', 'Mutsu,' and 'Pink Lady' (all seedlings of 'Golden Delicious') and 'Empire' and 'Fuji' (seedlings of 'Delicious'). Of 25 cultivars derived from crosses developed in Japan, nine (36%) are seedlings of 'Golden Delicious' (Bessho et al. 1993). Ironically, 'Braeburn', the newest sensation from New Zealand, is a chance seedling.

The introduction of resistance to apple scab incited by *Venturia inaequalis* (Cke.) Wint. by incorporating the *Vf* gene from *Malus floribunda* 821 has indicated the potential of interspecific crosses to introduce new genes (Crosby et al. 1992). The exploitation of naturally occurring mutations (budsports) in adapted cultivars has also been an important technique for improving apples. While most mutations are selected as improved color sports, mutations that change fruiting habit to compact spur-bearing farm have been very important in 'Delicious' and 'Golden Delicious'. The new technology of gene transformation promises to be the next technological breakthrough in apple breeding. This technique offers the possibility of introducing insect and disease resistance to established apple cultivars in a single

step, although thorough testing will be required. Sonic genetic transformants of apple are now in the testing stage.

MODERN BREEDING OBJECTIVES

The apple is grown as a composite tree consisting of a rootstock and a fruiting scion, and occasionally a three-part tree that includes a genetically distinct trunk or interstem. Thus, genetic improvement must involve both rootstock and scion. In the 1980s there was considerable revival of interest in planting self-rooted trees, a prospect made commercially possible by improved micropropagation methods. Loss of the growth-regulating chemicals necessary to control tree growth in the orchard has virtually eliminated interest in own-rooted apples but the concept is attractive for very high-density orchards.

Rootstock problems tend to be either catastrophic or debilitating. For example, fire blight of 'Malling (M.) 26' has caused huge losses in the United States, and the tree decline encountered in many plantings of the 'Mark' rootstock has caused serious economic problems worldwide. A new wave of clonal rootstocks, capable of surviving and thriving under a wide range of environmental conditions, inducing precocity, productivity, and good fruit qualities in the scion cultivar, is urgently needed in many parts of the world. In many areas of southern Europe there is almost universal use of 'M.9' rootstock, but a slightly smaller tree is considered desirable.

As a result of the long life of the standard orchards, only about 3% of bearing trees are replanted each year. Thus the cultivar picture in the industry has changed very slowly, despite the fact that there can be rapid change in newly planted cultivars. For example, 26% of all apples planted in Washington State in 1992 were 'Fuji'. Although marketers of apples tend to be conservative and prefer to deal with no more than a dozen cultivars, the appeal of new cultivars is very strong to growers because prices can be very high for new cultivars that have captured the imagination of buyers and the public, especially when the supply is limited. Nurserymen who sell trees are constantly seeking an advantage by controlling the source of new cultivars and of new sports of old cultivars. The objective of genetic improvement is to increase marketability of the fruit (or tree in the case of ornamentals) and to introduce traits that reduce production costs.

Increasing Marketability

Marketability of apple cannot be simply defined because of the many uses of the fruit. There is, in fact, not one market but many, e.g., fresh, stored, or processed; local market, commercial market or export. The market is dynamic, rather than static, and quality criteria vary. This may be a real problem if the breeder's concept of quality differs from that of the marketplace. The largest market is for dessert apples that are sold on the basis of appearance (size, color, shape, and freedom from blemishes) and quality (taste and mouthfeel). Apples are produced in a narrow

window (typically three months depending on maturity), but are consumed year round. Thus, a suitable cultivar that will become important in the industry must be able to be stored and have a suitable shelf life after storage. The world apple market has been reviewed by O'Rourke (1994).

Traditionally, the breeder has aimed at the "commercial market," now primarily large supermarket chains. There are two other major consumers of new cultivars, both serviced but casually by the breeder. the backyard fruit gardener (very important in Europe) and the direct-to-consumer orchardist. 'Freiherr von Berlepsch' and 'Ontario' have never made a place in the commercial market, but in Germany both are important for these specialty outlets. In the United States, scab-resistant cultivars have not yet made an impact on the commercial market but 'Liberty', 'Redfree', and 'Jonafree' are becoming increasingly important for hobbyists and organic growers; newer introductions such as 'GoldRush', 'Enterprise' . and 'Pristine' will enhance this trend.

It is clear that market targets must be identified by the breeder and that each market offers a valid niche that should be filled. But the breeder is responsible for introducing at least a rudimentary production system with each cultivar introduced. Such an orchard system should include identification of potential faults of the candidate and reasonable cultural practices to overcome these faults.

Fewer and fewer apple cultivars are destined exclusively for processing, although in Europe, special high-tannin cultivars of apples are used to make cider, an alcoholic beverage virtually unknown in the United States. Apple cultivars suitable for the processing market require heavy productivity, large size, with quality determined based on the processed product (typically high soluble solids, a specific sugar acid ratio, yellow flesh color, nonbrowning flesh, and an "apple" taste. Outward appearance is unimportant. There are also niche markets. These include small markets for very early fruits and apples with special attributes such as all-russetted types, unique flavors, and special size (from crabapples to oversized).

Conventional breeding has concentrated on quality traits. However, the definition of fruit quality is in some ways difficult to explain, even ephemeral. The problem is complicated because quality depends not only on genetic factors but is enormously influenced by production practices, orchard management, and climate. The traditional technique of increasing quality has centered therefore on improving size, appearance, and storage characteristics. In the 1920s and 1930s there were more that 40 apple breeding programs in North America and many more in other apple producing regions. These programs, based on intercrossing existing cultivars, have resulted in introductions such as 'Cortland', 'Empire', 'Fortune'. 'Fuji', 'Gala', 'Idared'. 'Jonagold', 'Macoun', 'Melrose'. 'Mutsu', 'Pink Lady', and 'Spartan'. Individual factors of quality will be discussed in more detail under Breeding for Specific Characters.

Regional preferences have had a tremendous effect on defining quality and determines which new cultivars will dominate the market. For example, the preference for 'McIntosh' in the northeastern United States is almost unique, not shared by the rest of the U.S. and European market and selections based on

'McIntosh' have been summarily rejected outside of this area. Similarly, Asian markets prefer mild to sweet, low-acid apples, while northern Europe and the U.S. Midwest appreciate tartness.

Appearance is also subjective. The market demands blemish-free apples, and any amount of russet (except for completely russetted apples, which still have a small niche market outside of the United States) is considered a serious defect even though it has no adverse effect on eating quality and in fact may have a positive effect. In the past, green skin was considered suitable only for cooking apples, but this has changed with the acceptance of 'Granny Smith'. The dessert market is now based on yellow, red, green, and pink, but other colors and color combinations are possible. Bicolor (or tricolor apples such as 'Cox's Orange Pippin') are popular in Europe, especially yellow apples with a distinct red blush, and red color over a yellow ground color. Red and green or red over green which leads to a dark "liverish" color typical of some 'Delicious' strains, is considered unsuitable in Europe. The United States prefers washed color while Europe prefers striped apples.

Fruit shape is important. Ovate or conic apples are preferred; globular apples are acceptable, but flat, oblate apples are now considered unacceptable (for no rational reason). The "typey" shape of the ovate 'Delicious' with a lobed calyx end is highly acceptable for this cultivar, and special sprays (gibberellin and auxin) are applied in the eastern and midwestern United States to obtain suitably shaped fruit.

In general, the preference is for large apples, 70 to 85 min (2 3/4 to 3 1/4 inches) in diameter; in Japan even larger apples are preferred. Apples smaller than 57 mm (2 1/4 inches) in diameter, often termed "schoolboys" in the United States, are unacceptable; apples below this size are called crabapples and have essentially no value except for juice. Of course, many consumers prefer small apples, but this is not reflected in the market price. Clearly, a special marketing and packaging program for small apples is needed.

Reducing Production Costs

Introducing traits that reduce production costs is another way to achieve profitable apple growing. One strategy is to achieve high yields through a combination of adaptability, precocity, productivity, and harvest consistency from year to year. Production costs can also be reduced by increasing the case of harvest (through dwarfing rootstocks) and disease and insect resistance. Apples that are deficient in these qualities can still be grown but must be "propped up" by cultural practices, many of which are expensive and some of which are considered to be undesirable for the environment and public health. For example, 'McIntosh', an apple with unusual taste qualities, suffers from early drop, soft fruit, and poor color. For many years this problem was solved by the grower with preharvest spays of an anti-gibberellin growth regulator daminozide, sold under the trade name Alar, which kept the apple on the tree to increase color without loss of firmness. This chemical prop was lost when daminozide was banned after high publicized but unsubstantiated claims that it was carcinogenic. Although 'McIntosh' has not disappeared, new

plantings have sharply declined and the cultivar in being replaced by 'Empire' and other new cultivars. Attempts are underway to transform McIntosh' with genes to reduce ethylene accumulation to diminish this problem.

In marginal areas of apple production, breeding is essential to obtain adaptability. Objectives include cold hardiness for climates with extreme winters, low chill requirements for subtropical climates, and sunbwn resistance and/or very late ripening for hot, and climates. Genetic disease and insect resistance we also required for *those areas* where other means of control are nonexistent, unavailable, too expensive, or unacceptable. Disease-resistance breeding has been an important strategy to obtain adaptability and reduce production costs.

The consuming public buys apples on the basis of name, appearance, quality (so far as can be judged), and price. While apples that can be produced more efficiently might presumably be sold at a lower price, the consumer has no way of knowing this. The breeder should be aware that apples with pest resistance, while of interest to growers and to hobby and home orchardists, have had, until recently, little interest to the average consumer and thew apples must compete with susceptible apples an a strict quality basis. However, some consumers have become interested in "organic" apples, fruit that is presumably grown without certain "nonorganic" pesticides, and disease-resistant apples could become a potential selling point for this market. The bottom fine, however, is that whatever the improvement, the final competition of apples at the marketplace is based on fruit characters alone.

BREEDING TECHNIQUES

Floral Biology

The flowers of apple cultivars and seedlings vary considerably in size and petal shape, and in color from white to deep pink. They are produced at the same time as the leaves, and we borne in cymose clusters on fairly short pedicels, usually on spur type growth, but in some instances from the termirial or lateral buds of the previous season's growth. The typical flower consists of five petals, a calyx of five sepals, about 20 stamens and the pistil which divides into five styles. The ovary has five carpels, each usually containing two ovules, so that in most cases, the maximum seed content is 10 but some cultivars have more. 'Liberty' and 'Northern Spy', for example, usually have 12 to 18 seeds and the 'Ottawa 3' rootstock often has 20 to 30 seeds.

Pollination

Pollination is the mechanical transfer of pollen from the anthers to the stigmas and is a prerequisite to the fertilization of the ovules and the development of seeds and fruit. Most apples require cross-pollination, which necessitates the transfer of pollen

from the flowers of one tree to the stigmas of a genetically different one. In the orchard, pollination is carried out by insects, particularly bees, in their search for pollen and nectar. Most flowers are admirably designed to facilitate this process, but in a few genotypes, such as 'Delicious', the stamens are long and the styles short, which allows the bees to visit the flowers without making contact with the stigmas. This character may lead to a considerable loss of the potential crop.

Pollen collection. The collection of pollen is the first part of the hybridization process. Flowers are collected at the balloon stage just before the petals expand, and before the anthers dehisce, although circumstances often demand that they are collected a little earlier. Anthers are removed by rubbing flowers over a screen and are deposited into petri dishes or similar containers to dehisce. Putting the collected anthers in a paper "boat" and leaving them overnight under an incandescent lamp is a satisfactory method. The pollen once dry can be used immediately. Since not all cultivars flower at the same time (weeks my elapse between the flowering of the earliest and the latest), pollen may be collected from the earlier parent and stored until required. Pollen may be forced in the greenhouse by cutting flowering shoots and leaving them in a vase with water. Dried pollen can be conveniently mailed or dispatched in polyethylene packets, making it easy to share germplasm. Pollen in loosely stoppered vials placed in a desiccator containing calcium chloride can be stored for a year or more at $-15°C$. Apple pollen can be preserved longer at cryogenic temperatures (Hanna and Towill 1995).

Viability can be estimated microscopically after staining with acetocarmine (Marks 1954) or germinated on a drop of sugar solution (2.5 to 20%) to calculate germination. Most recommend 10% sucrose in aqueous solution or 5% agar at $20°C$ for good pollen tube formation. The addition of 10 ppm boron to the medium increases germination (Thompson and Baijer 1950).

Emasculation. Before crossing, the flowers of the seed parent are usually emasculated at the balloon stage. Removal of the petals in the emasculation process serves three purposes: (1) it prevents self-pollination (although self-incompatibility makes this a rare occurrence); (2) it exposes the stigmas to facilitate cross pollination; and (3) it minimizes visitation by bees and pollinating insects attracted to the petals, reducing contamination with unknown pollen. When hybridizations are being made for genetic studies stricter precautions are needed to prevent contamination, and emasculated flowers are often bagged. If crosses are part of a breeding program with the sole purpose of producing improved cultivars, then 100% control is not essential. Emasculation is usually achieved by flicking off the sepals, petals, and stamens with the fingernails, which is easiest at the balloon stage. Only the styles are left sticking up from the receptacle and ovary. Other emasculation methods include the use of scissors with a notch cut in the blade (Barrett and Arisumi 1952). At Cornell University, Geneva, pollinations are usually made immediately after emasculation and again a day or so later, especially when weather conditions are marginal.

Apple trees produce flowers in grew abundance and only a small proportion of them can set and produce fruit. Typically, two flowers per cluster are emasculated and the others removed. Unemasculated flowers producing fruits can be identified, because fruits from emasculated flowers have no calyx. It is convenient to emasculate first, leaving an unemasculated flower as a "flag" to identify the cluster, this flag is removed at the final pollination. Experiments have shown that insects do not visit flowers when the stamens and petals have been removed, thus there is no need to protect them from insects (Visser 195 1).

Other techniques can be utilized to achieve cross-pollination. One is to eliminate emasculation entirely to avoid injury, but to bag flowers (pollinated in the balloon stage after manually opening the petals), to prevent visitation from insect pollinators. The crossed cluster must be tagged because the telltale "belly button" appearance of the calyx end of crossed fruit derived from emasculation is lacking. This technique is common in Europe. Another technique is to enclose a seed tree in bee-proof screening and to enclose a bouquet of open flowers of the desire pollen parent and insert a small package of honeybees. But if too many bees are provided they will harvest pollen form the target stigmas and lower fruit set. Another approach is to provide bouquets of a single cultivar that contains a dominant "marker" gene that can be identified in the seedling stage in an orchard of several cultivars. For example, if bouquets of a disease-resistant apple such as 'Liberty' or 'GoldRush' are placed in an orchard of 'Fuji' and 'Gala', seeds collected from trees close to the bouquet could be expected to have a small percentage of the appropriate hybrid that could be identified by screening in the seedling stage. A final technique is to collect open-pollinated seed from orchards that contain two cultivars of interest. For example, 'Empire' was selected from open-pollinated seeds obtained from an orchard containing only 'McIntosh' and 'Delicious' in a year when all hand pollinations failed.

Crossing. Pollination can be achieved by dipping a small, soft brush into a vial containing the pollen and lightly brushing the stigmas. The brushes can be cleaned by immersing them in 95% alcohol and drying them before applying a new pollen. Another technique is to dip the eraser end of a pencil or the fingertip into the pollen and touch the stigmas to transfer the pollen. The finger technique uses a lot of pollen but the pollinator can see the yellow pollen and can feel the touch of the stigmas. Either way is quick and effective. Some pollinate flowers immediately after emasculation; others prefer to emasculate and return in a few days and make the pollinations when the stigmas are more receptive. It is best to avoid pollination when temperatures are below 50°C because pollen tube growth may be inhibited. The whole tree may be pollinated with one parent or a number of crosses may be made on different parts of the tree, providing limbs are tagged carefully to prevent mix-ups.

In an effort to improve seed set and seed germination, Keulemans et al. (1994) examined the influence of the number of pollinated stigmas per flower, the number of flowers per cluster, and pollinator. Seed set increased with the number of pollinated stigmas on the flowers. Compared to one flower per cluster, seed set was slightly

reduced when three flowers per cluster were left. The pollinator had no clear effect on final fruit set, but genotypic effects were noted. For all pollinators, seed germination was negatively correlated with seed number. A delayed harvest reduced seed germination.

Seed Handling

Crossed fruit should be harvested slightly before it ripens. Dry seeds can be stored if they are not to be planted in the following year. Seed storage has been reviewed by Ellis (1985). Apple seeds will not germinate unless stratified. This involves keeping the seeds in moist conditions and subjecting them to a period of cold to allow after-ripening, during which embryo changes occur. The easiest technique when seeds are not stored is to leave seeds in the fruit and store them in cold but above freezing temperatures where they stratify naturally, but moldy core can cause problems. Seeds should be removed from any damaged or rotting fruit at harvest. The usual technique in most breeding programs is to extract seeds slightly before fruit maturity. This avoids losses due to pilfering or mix-ups due to dropping—a problem when more than one cross is made on a tree. At Cornell University, Geneva, individual fruits are labeled well before ripening time on trees in which several pollens have been used. After-ripening will proceed at temperatures between 0 and 10°C, but the optimum is from 3 to 5°C. The time required may vary from 6 to 14 weeks and depends to some extent on the temperature. Abbott (1955) has shown that the temperature for after-ripening is critical. Above 17°C the after-ripening process reverses and the longer seeds are held above this temperature the longer the period of cold required to allow them to germinate. Seeds can be stratified in polyethylene bags containing moist filter paper or moistened peat moss, which is slightly fungistatic. The stratification process is usually completed in 6 weeks but should be checked periodically for radicle emergence. When 50% or more of seeds have germinated they can be planted in seed boxes or trays with individual cells, and placed under optimum conditions for seedling growth.

Rots may be troublesome during stratification, particularly if the seeds are not clean when put in the trays. *Rhizoctonia solani* Kuhn, the principal culprit, grows on the outside of the testa and in due course penetrates and causes the embryo to rot, quickly spreading from seed to seed. It is always advisable to surface sterilize the seeds in calcium hypochlorite solution (10 g in 140 mL water and filtered) for 5 min, and then wash them before stratification. At Cornell University, Geneva, seeds are soaked overnight in a saturated suspension of Captan fungicide before stratifying and a second fungicide treatment is often necessary after planting.

Juvenility

Apple trees undergo different phases of development between seed germination and the adult fruiting tree. In the juvenile phase, when no flowers are produced, plants may differ considerably from mature adult trees. The leaves are smaller and

usually more finely serrate, and the shoots are thinner and are often produced at right or obtuse angles to the main stem. Bud break is early and leaf fall is late. The onset of the adult phase is marked by the development of flower buds. Between these two phases there is a transition phase when the lower part of the plant is still juvenile and the upper part is adult. Cuttings from most adult apple trees used as scion cultivars are extremely difficult, if not impossible, to root. Cuttings from young seedlings root readily but rooting is more difficult to achieve as the seedlings age, suggesting that the transition is gradual. The duration of the juvenile phase varies from 3 to 10 or more years, depending on the genotype of the seedlings and the cultural practices.

Ways of shortening the duration of the juvenile phase have been investigated (Kemmer 1953; Murawski 1955; Visser 1964). Methods attempted to induce adult trees to flower include shoot priming, root priming, and bark ringing. However, any method that restricts growth in the very young seedling does not shorten the duration of the juvenile phase but in fact lengthens it and delays the onset of fruiting. Cultural methods that check the growth of seedlings are effective in hastening flowering only when a certain stage of development has been reached. Way (1971) was able to induce seedlings to flower by bark ringing when they were 4 years old, but 3-year-old seedlings did not respond.

Other cultural methods can effectively shorten the juvenile phase. One is to grow the seedling as fast and vigorously as possible. This can best be achieved by avoiding any check to growth in the early stages of development and, where this is not possible, to keep disturbance to a minimum. Seedlings have flowered in the greenhouse in I year by keeping them in a continuous state of growth (Aldwinckle et al. 1976). Seedlings grown under optimum conditions in the greenhouse, closely planted, watered, and fed, may reach 3 m in height in the first season compared with I in for seedlings planted out in the field. These may be planted directly into permanent field locations. The nursery should be avoided because it delays the process. Seedlings should continue to grow freely and, since the seedlings first come out of the juvenile condition at the top of the seedling, they should be left unpruned until they flower and fruit (Brown 1964; Zimmerman 1971). Tydeman and Alston (1965) have shown that the juvenile phase can be considerably shortened by budding seedlings onto dwarfing rootstocks. Buds were taken from the upper part of the main shoot in late surnmer of the second year's growth and worked on the dwarfing rootstocks 'MY and 'M.27', grown as closely planted cordons with all lateral growth pruned back annually in late summer. Nine years after germination 88% of 902 budded seedlings had fruited compared with 49% of those on their own roots. Virus-free rootstocks must be used to avoid infection.

Juvenility poses a different sort of problem for the rootstock breeder. In the stoolbed, juvenile plants are usually quite thorny, root very easily, and exhibit earlier bud break and later leaf fall. Evaluations in the rootstock program at Cornell University (Geneva Station) are usually made in the third season, when these juvenile characteristics have somewhat abated.

Seedling Selection

As soon as the seeds have germinated and the first true leaves are showing, the long process of seedling evaluation may begin. Some of this selection occurs naturally. A *pale green* lethal recessive is carried in 'Golden Delicious', 'M.9', and many other cultivars (Klein et al. 1961) and 25% mortality can be expected when these cultivars are intercrossed. Negative decisions should be made as early as possible because every delay in decision-making adds to the costs of eventual introductions. Even more important, delay limits the attention that should be given the best candidates and so limits the numbers of such elite candidates that can be evaluated (Cummins and Aldwinckle, 1988).

The seedling stage is the best time to select for resistance to diseases where infection occurs on young leaves, such as scab and cedar apple rust (*Gymnosporangium juniperi-virginianae* Schw.). Inoculations with spore suspensions at this early stage show infections on the young leaves in a few weeks and the susceptible seedlings can be discarded. Selection for scab resistance alone can reduce the size of the progeny by 50 to 80% depending on selection criteria. Fire blight inoculation of seedlings 50 to 90 cm tall with strains of *Ervinia amylovora* (Burrill) Winslow et al. will eliminate a large percentage of most progenies. Some other diseases such as mildew (incited by *Podosphaera leucotricha* [Ell. & Ev.] Salm) are better left until the seedlings are in their second year of growth before being assessed for resistance. It is important to be able to discard inferior seedlings at the earliest possible stage of growth.

Many growth characters can be evaluated in the early years of development of the seedlings, such as tree weakness, the production of long spindly shoots, and other defects in general growth that would prevent the seedling from ever making a satisfactory tree. Any such defect should be enough to eliminate the seedling. Some correlated characters can also be detected, such as late leafing, which is associated with late flowering, and early selection can be made when this is one of the breeding aims. Similarly, the rootstock breeder can use very early screening for susceptibility to *Phytophthora* spp., to fire blight, and to woolly apple aphids to reduce the original population of seedlings by 95% before commencing any horticultural evaluations.

Preselection

Searches are always being made for characters in the juvenile stage that are correlated with fruit characters in the mature tree. Any positive correlation would be of considerable value in allowing seedlings with undesirable fruit characters to be discarded at an early stage. Such correlations must fulfill two requirements: (1) The degree of correlation must be of a fairly high order, and (2) the character in the juvenile plant must be reasonably easy to recognize, for any time-consuming series of measurements or complicated chemical analyses greatly reduces the value of the correlation as a practical means of preselection.

A number of correlations have been claimed but few are of any value to the breeder. Nybom (1959) found a correlation between the pH of the leaf sap and the pH of the fruit juice, with leaves of the sweet type at pH 5.7 and the acid types 5.5 to 5.6. This has not proved to be a reliable method because the differences are so small that the error between samples from the same seedling are often greater than between seedlings. Another correlation that is often claimed is between red fruit color and anthocyanin pigmentation of other parts of the tree such as 1-year-old shoots or leaf petioles. The distribution of anthocyanin in the tree depends on so many factors and there are so many exceptions to these correlations as to make this of no value as a method for predetermining fruit color. Only in progenies from trees that are pigmented throughout the tissue with purplish red anthocyanin, as in *Malus pumila niedzwetzkyana* Schneid., can red-leafed seedlings be selected that will produce trees with red leaves and shoots and fruit with purplish-red skin and flesh. Some useful correlations have been established between late leafing and late flowering, between length of juvenile period and fruiting age in the vegetative propagules, and others that will be discussed under the appropriate character headings. Selection for tree type might reasonably be expected to be selected for in the early seedling stage but experience with evaluation of progeny of 'McIntosh Wijcik', an extreme, highly spurred mutant heterozygous for the dominant *columnar* gene (Co), indicates that this is dangerous. In the future, preselection will be made by associating important traits with molecular markers. This is discussed in greater detail under Biotechnology.

Fruit Evaluation

When the seedlings produce flowers and fruit, the most important part of seedling evaluation begins: assessment for fruit quality. Increasingly, researchers are looking for methods of quality assessment that will correlate with consumer preferences (Redalen 1988). There are many criteria by which the fruit can be judged; to fall below the standard in any of these will automatically eliminate the seedling. Size is one that can be easily assessed. Fruits consistently below 60 min in diameter are too small for a commercial dessert apple. Color is important, with preference for clear yellow, bright red, bright green, or bicolor fruit. These should be free from russet, although there may be a place for sour completely russet apples. Dull-looking apples are not worthy of further consideration. Shape has become important. Irregularly shaped apples and especially very oblate apples will not be accepted by marketers. Flavor is of prime importance. Many seedlings have fruit with very little acid and in consequence they are very sweet and insipid. This type is unacceptable except in some Asian markets. Fruit may have a strongly aromatic or distinct anise-like flavor and, while these may prove to be good home garden apples, they are unacceptable as commercial apples because such flavors are not universally appreciated. The ideal is sub-acid with a pleasant flavor but there is increasing demand for apples with complex, spicy, and full flavor. Of particular importance is the flavor after storage. Apples that develop an aldehyde or off-flavor will be unacceptable. Apples that are too mild will often taste bland after storage because

acidity decreases. The texture and mouthfeel of the flesh should be firm and crisp with plenty of juice. Crisp flesh should persist in storage; soft apples are unacceptable. Overly thick skin can be a problem as well as skin that is too sensitive to bruising, such as 'Sir Prize'.

When the fruits of the seedlings have been examined and those that meet all the requirements have been selected, the next stage of selection is for yield. Care must be exercised to be sure that each selection is kept virus-free. For this a number of trees need to be propagated on commercially important rootstocks and grown in properly conducted trials with the best standard cultivars as controls and crop weights determined and grade distribution assessed. At the same time, the selection can be topgrafted on an existing tree so that fairly large samples of fruit can be obtained and be subjected to storage trials and examined for susceptibility to storage diseases. Where possible, the selected seedlings that show real promise should also be grown in trials in a number of localities to see if their behavior is consistent under varying conditions. In these trials, only those selections with heavy annual yields need be considered further.

During the selection process it is important to be absolutely ruthless and to eliminate from further consideration any seedling that does not reach the required standards. Carrying out trials on selected seedlings that never make the grade is a waste of time and money. No seedling is perfect in every character but the breeder needs to strike a balance between eliminating everything and releasing too freely. Because apples may perform differently in different locations, wide testing of promising seedlings is essential.

Records

An important part of any breeding program is record-keeping. What records are kept and how detailed they are depends entirely on the amount of information required for future use. Descriptor lists for apple are available (Watkins and Smith 1982). Selection indices based on a number of characters have been proposed by Blazek and Paprstein (1988). It is always tempting for the breeder to engage in genetic studies, but many breeders have become bogged down with record-taking at the expense of moving vigorously forward to the goal of cultivar improvement. In general, it is more efficient to carry out genetic studies on specific crosses because extracting voluminous data from all crosses is inefficient. There are a number of ways of making field records. One convenient system is to use a predetermined numerical classification, typically on a I to 5 or 0 to 9 scale to save time and space. Verbal descriptions are not amenable to computerization. The details of a seedling can be contained in one line across a scoring sheet.

Mutations and Chimeras

A bud sport is a mutation arising in a cell from which a bud develops. It produces a shoot that differs, usually in only one character, from the plant on which it was

produced. These mutations can affect any part of the plant. There are two important types of mutations in apple: (1) those that produce single gene differences in some character of the tree or fruit, and (2) those that alter ploidy. Mutations affecting the appearance of the fruit are easily recognized and are the type most often found. Increases in the amount of anthocyanin in the outer cell layers of the fruit skin are most common and red sports of many of the popular cultivars have arisen. Some cultivars such as 'Cox's Orange Pippin', 'Delicious', 'Estar', 'Gala', 'Jonagold', 'Rome Beauty', and 'Winesap' are prone to produce mutants of this type, while other widely grown cultivars are stable and seldom seem to mutate. Not all the red sports of one cultivar are necessarily identical; apart from differences in the area of the fruit covered, the intensity of the pigmentation in the outer cell layers can differ (Dayton 1959). Very often the mutation is limited to one cell layer in the apical meristem; therefore, the plant is likely to be a periclinal (hand-in-glove) chimera. The mutation is usually not heritable unless the second layer (L-2). which gives rise to gametes, is involved (Pratt 1983). Bud sports with increased red color may be extremely valuable and should be propagated and trialed to see if they are superior to the original clone. Reduced russet, russet-free, and total russet occurs and some of these traits may be desirable.

Mutations affecting growth habit, particularly the spur or compact types, which produce compact or dwarfish, freely spurring trees, may be extremely valuable and are being sought in all the important commercial cultivars. Mutations inferior to the original clone my also occur and stock trees should be carefully observed so that no inferior mutants are unwittingly propagated. This is the basis for bud selection to maintain the integrity of the cultivar.

The rate at which single gene mutations occur can be increased by irradiation with X-rays, gamma rays, or thermal neutrons. Bishop (1959) produced two dark red sports of 'Cortland', two sports of 'Sandow' with less color and more russet, and a 'Golden Russet' with considerably less russet than normal. Lapins (1965) and others have produced compact mutations by means of irradiation. The effective dosage is 3-5 krad for dormant scions and 2-4 krad for summer buds when X-rays are used and 3.9-15.6×10^{12} thermal neutrons/cm^2. Mutants from irradiated material do not readily show themselves and the normal growth if allowed to develop will suppress the mutant. When dormant scions have been treated and grafted, the basal ten buds or so of the shoot emerging from each treated bud should be removed and budded onto a dwarfing rootstock. The mutants are likely to show in the second vegetative generation. Lacey and Campbell (1987) reviewed the production and selection of mutant apples and described the tests and the experimental evidence used to determine the nature of the mutated plants.

The other mutations important in apple breeding are the "large" or "giant" sports (Einset and Imbofe 1947, 1949, 1951) due to changes in ploidy level, typically chromosome doubling (diploid to tetraploid or triploid to hexaploid). These sports are usually recognized first by their large f1ruits, which are sometimes twice as large (in volume) as their diploid counterpart. The fruits, apart from the increase in size, are usually flatter and more irregular in shape and have no commercial value.

Giant sports of a number of our most widely grown apple cultivars have arisen

spontaneously and have been discovered by observant growers. Most of these tetraploids are periclinal chimeras and can be grouped according to the arrangement of the $2x$ and $4x$ tissues. The four layers in the apical meristem (L-1 to L-4) can be classified on the basis of nuclear size as either 2-2-2-2, 2-2-2-4, 2-2-4-4, 2-4-4-4, or very rarely 4-4-4-4 based on whether the cell is diploid or tetraploid. The breeding behavior of these sports differs according to the location of the 4x tissue. Only when L-2 is $4x$ will the tree breed as a tetraploid. Thus many giant sports, apart from having tetraploid growth characters, genetically behave as diploids. The 2-2-4-4 cytochimeras look like a tetraploid but breed as a diploid (Pratt 1983).

It is possible, however, to produce homogeneous tetraploid plants from 2-2-4-4 sports by inducing shoots to grow from endogenous tissue or from root suckers developed from the scion because as roots develop from L-3. One method is to grow 1-year-old trees of the giant sports in large containers, cut them down to 30 cm, and remove all the buds and any growth that appears in the region of the removed buds or from the rootstocks. This encourages the formation of sphaeroblasts in the internodal regions. These will in due course crack and produce adventitious buds which develop into shoots. Having been developed from the region of the phloem, these shoots should be 4-4-4-4. Although practically all the $4x$ sports have arisen spontaneously, it is also possible to encourage the formation of tetraploids by the use of colchicine applied to the growing point (Hunter 1954). These induced tetraploids are also likely to be cytochimeral.

BIOTECHNOLOGY

Micropropagation

Information on tissue culture of apples has been reviewed by Zimmerman (1984, 1991) and Skirvin (1986). Micropropagation using shoot proliferation has been achieved by standard protocols. Shoot proliferation medium is based on Murashige and Skoog salts with minimal organics, and the addition of cytokinins such as 4.4 µM benzylamino purine (BA) or 0.1 µM thidiazuron, the inclusion of 0.5 µM indolebutyric acid (IBA) and 1.4 µM gibberellic acid (GA) and 87.6 mM sucrose using agar as a gelling agent. Rooting medium is modified by halving the concentrations of salts and sucrose, eliminating BA and GA, and varying the concentration of IBA. A simpler method is to place shoots for 3–7 days in the dark in a liquid medium containing 43.8 mM sucrose and 1.5 µM IBA at 30°C. (Zimmerman and Fordham 1985). Protocols vary with genotype and many are proprietary.

Tissue culture has been seen as a means to propagate fruiting cultivars on their own roots but results have been variable. Own-rooted apples trees are vigorous and branch freely. Flowering may be delayed as compared to planting budded trees (which are usually older). Many-branched nursery stock will produce a crop in the second leaf. Tree size of own-rooted trees will obviously vary with each genotype.

Apple rootstocks have been micropropagated in Canada, Italy, France, and the United States, but cost is not competitive with stoolbed production. Thus

micropropagation has been used to establish new stoolbed plantings with disease-resistant clones. It is essential to establish new cultures annually to assure that no genetic change has occurred during the micropropagation process. The chance of mix-up or genetic change is ever-present and some nurseries have avoided micropropagated rootstocks.

Somatic Embryogenesis

Embryogenesis has been induced from nucellar tissues from seeds (Eichholtz 1979) and calli from various in vitro-grown apple seedlings or from flower buds and excised petals from field-grow trees (James et al. 1994). However, there have been no reports of complete development of viable seedlings from adventitious embryony.

Embryo Rescue

Development of immature embryos (8 days following pollination) have been achieved (Zhang and Lespinasse 1988), but rescue of interspecific or intergeneric: hybrids has not been attempted.

Protoplast Culture

Viable protoplasts have been isolated from shoot cultures of shoots, callus, and cell suspensions of various apple genotypes and rooted autotrophic plants were reported from 'MY and 'Spartan' (Patat-Ochatt et al. 1988). Wallin and Johansson (1989) regenerated shoots of a columnar apple (A 1583) from leaf mesophyll protoplasts. There are no reports of successful protoplast fusions and subsequent regeneration in apple.

Regeneration

An efficient regeneration system is a prerequisite for apple transformation (Skirvin et al. 1986). Important factors that influence regeneration include regeneration media, age, genotype and orientation of the explant; and incubation conditions (James et al. 1988; Welander 1988; Fasolo et al. 1989). Regeneration has been obtained from shoots and leaf segments (Welander 1988), leaf disks (Elobeidy and Korban 1988), and leaf strips (James et al. 1988). Leaves are most commonly used because of their abundance and ease of manipulation for *Agrobacterium* infection (Jams and Dandekar 1991). For maximal transformation and regeneration efficiency of apple leaf explants, the shoot culture must be between 20 and 40 days of age (Bondt et al. 1994). Hyperhydricity (vitrification) must be avoided. Exposure to darkness for I to 4 weeks induces regeneration (Welander 1988; Fasolo et al. 1989; Korban et al. 1992, Durham and Korban 1994).

Prior to transformation, the effect of aminoglycosides on regeneration and growth of explants must be determined because cefotaxime is used in transformation studies to control growth of surviving *Agrobacterium* after infection. The stimulatory effect on morphogenesis has been observed in apples (Maheswaran et al. 1992).

Molecular Markers

Biochemical markers such as isozymes have been extensively studied in plants and many important agricultural characters have been correlated with isozyme polymorphism (Weeden 1989). As a result of the high heterozygosity and high level of polymorphism that exist in apple, isozyme techniques have provided a reliable method for cultivar identification in scion and rootstock (Vinterhalter and James 1983, 1986; Weeden and Lamb 1985, 1987; Menendez et al. 1986; Weller and Costante 1987; Manganaris and Alston 1989; Samimy and Cummins 1992). Evidence for the allopolyploid nature of the apple genome was demonstrated using apple pollen enzyme system (Chevreau et al. 1985). Evidence that the maternal parent generally provides the diploid gamete in natural triploids was demonstrated with the isozyme 6-phosphogluconate dehydrogenase (Chyi and Weeden 1984).

Genetics and linkage analysis of isozymes has been studied in apple (Weeden and Lamb 1987; Manganaris 1989) and isozymes have been used in the development of linkage maps to anchor homologous linkage groups and to establish marker points (Hemmat et al. 1994). Isozyme *ldh-2* has been shown to segregate with fruit color on group 3 of the apple linkage map (Weeden et al. 1994). A close linkage was demonstrated between the genes for endophosphatase (*ENP-1*), acid phosphatase (*ACP-1*), and the pale green lethal (*l*) (Manganaris and Alston 1988).

A number of commercially important traits have been linked to isozyme loci. Self-incompatibility has been linked to *GOT-1* (*Aat-c*) (Manganaris and Alston 1989), and scab resistance (*Vf*) is about 8 cM from *PGM-1* on the apple linkage group (Manganaris et al. 1994). Powdery mildew resistance has been identified by two closely linked isozyme markers ACP-3 and *GOT-2* (*Aat-p*) (Manganaris 1989). Although 19 isozyme markers have been mapped on the apple linkage map, the method has its limitations. Those isozymes that show a well-defined metabolic role and have highly conserved isozyme numbers are particularly useful (Weeden 1989). However, the number of isozymes that can be assayed is limited. Most of them are monomorphic and are of limited value for gene tagging.

The development of polymerase chain reaction (PCR) techniques has provided a new method of detecting DNA polymorphism by amplifying specific DNA fragments. Random amplified polymorphic DNA (RAPD) fragments have been useful and reliable for genetic mapping and gene tagging in many different plant species including apple (Weeden et al. 1992). Decamer random oligonucleotide primers are usually used in this method (Williams et al. 1990). The advantage of the RAPD technique is that it can provide numerous genetic markers in a short time, making it the method of choice for mapping economically important plants. A genetic link

age map constructed from over 400 markers, mainly RAPDs has been developed for apple based on a cross of 'Rome Beauty' with 'White Angel' Olemmat et al. 1994). Maps from other progenies are under development (Conner et al. 1995; King 1995). In a study of two half-sib apple progenies, Conner et al. (1995) reported on the usefulness of molecular marker-bail association in different genetic and environmental backgrounds. Approximately half of the marker-trait associations were detected in both progenies.

RAPD markers can be very useful for cultivar identification. Landry et al. (1994) used RAPD marker data in phylogeny analysis of 25 apple rootstocks and developed a DNA fingerprinting system and identification key for apple rootstocks. Koller et al. (1993) reported 14 RAPD markers resulting from two decamer primers could discriminate 11 apple cultivars.

RAPD markers arc proving useful in plant breeding (Williams et al. 1990; Welsh and McClelland 1990). They enable apple breeders to tag any genome region early in the seedling stage (Weeden 1989). One important advantage of this technology is that a genetic map is not necessary in order to identify a trait of interest. The correct selection of a cross for identification of a particular trait, however, is an important factor in the search for markers. Bulked segregant analysis (BSA) is the quickest approach in identifying markers in any population that a gene or a trait of interest is segregating (Michelmore et al. 1991). Using the BSA technique in apple, several tightly linked RAPD markers were identified for the scab-resistance gene (*Vf*) derived from *Malus floribunda* 821 by various groups (Durham and Korban 1994; Koller et al. 1994; and Yang and Krilger 1994; Hemmat et al. 1995, Tartarini, in press). S. Tartarini (personal communication) using several RAPD markers close to the *Vf* locus determined the portion of the original *Malus floribunda* 821 DNA present in each of 30 cultivars.

A map of the scab resistance region has been developed (Hemmat et al. 1995), showing three RAPD markers closer to the *Vf* loci than the isozyme marker PGM-1. These markers could be of great value for resistance selection and screening. Many other morphological and developmental traits, such as terminal bearing, reproductive bud break, bloom time, and root sucker formation, have been mapped within several centimorgans of a RAPD marker on the apple linkage map (Lawson et al. 1995). Markers for anthocyanin fruit color have been identified and demonstrated to be present in many red apple cultivars (R. Cheng, unpublished). A RAPD marker for the columnar habit (Co), which suppresses branching, was found to contain a simple sequence repeat (SSR) that is highly polymorphic in apple (Hemmat et al. 1995). This SSR has been napped on linkage group 11 of the 'Rome Beauty' × 'White Angel' linkage map. A marker (*OPAT20*) for powdery mildew (*Podosphaera leucotricha*) resistance has been reported (Markussen et al. 1995) and was mapped within a genetic distance of 4 cM from P*11* gene from *M. × robusta*. This marker could provide a convenient method for selection of the resistant genotype in progenies. Other complex traits such as branching habit, flowering time, vegetative bud break, and acidity and the genetic basis of these characters are under investigation using molecular markers and isozymes that offer great potential for assisted selection in quantitative traits (Hagens 1992; Conner et al. 1995).

Transformation

Apple transformation was first reported by James et al. (1989) based on *Agrobacterium*-mediated transformation of leaf disks of 'Greensleeves' using the binary vector pBin6. Detailed transformation protocols have been published by James and Dandekar (1991). However, *Agrobacterium* transformation systems must be optimized for each crop species and indeed for each cultivar. Explants are taken from the plants established in culture and co-cultivated with *Agrobacterium* carrying the vector plasmid. Transgenics are regenerated under selective conditions. Although transgenic apple plants have been produced, until recently efficiency has been low, even with highly regenerable cultivars (James et al. 1993a,b), but rates of 20% or more have been achieved with several cultivars (H.S. Aldwinckle, personal communication). Important factors that influence transformation include pre-culture conditions, the strain of the bacteria used, wounding of leaf tissue prior to bacterial treatment, induction of the bacteria, co-cultivation conditions, explant, genotype, the regeneration system, and conditions to select out transgenics (James 1991; James and Dandekar 1991; Welander and Maheswaran 1991; Bondt et al. 1994). The transgenes incorporated into apple via *Agrobacterium*-mediated transformation are stably incorporated and inherited in a Mendelian fashion (James et al. 1994).

In apple, several genes have been targeted for transformation using *Agrobacterium*-mediated gene transfer, including those for insecticidal crystalline proteins (ICP) from *Bacillus thuringiensis* and the cowpea. trypsin inhibitor protein (cpTi) (Ely 1993; James et al. 1993a); lytic peptides for bacterial disease resistance (Norelli et al. 1994); antisense genes for reduction of softening (Gray et al. 1994; Yao et al. 1995); cross-protection for viral resistance (Mauren et al. 1992); and chitinase genes for fungal resistance (H.S. Aldwinckle, personal communication). In apples, qualitative genes for resistance to major pests are available and are potential targets for future improvements (Brown 1992). These include several dominant genes for resistance to apple scab, *Venturia inaequalls* (Shay et al. 1953); resistance to mildew, *Podosphaera leucotricha* (Knight and Alston 1969); and resistance to woolly apple aphid, *Eriosoma lanigerum*. The *Co* gene that confers columnar plant habit is also desirable (Lapins and Watkins 1973). Techniques are available to isolate, identify, and introduce the disease resistant genes into existing cultivars (Michelmore et al. 1992a,b; Michelmore 1995; Martin 1996). Cultivars transformed include 'Greensleeves' (James et al. 1989), 'Delicious' (Sriskandarajah et al. 1994), 'Royal Gala' (Yao et al. 1995), 'McIntosh' (Bolar 1995), and the rootstocks 'M.26' (Lambert and Tepfer 1992; Maheswaran et al. 1992) and 'M.7' (incorrectly reported as 'M.26') (Norelli et al. 1994).

The incorporation of insecticidal activity of proteins made by *Bacillus thuringiensis* would be an effective strategy to control codling moth, one of the major pests of apple. Chemical control of secondary infections of codling moth also kill predators of mites and aphids; thus genetic control of codling moth could have a major effect on other apple pests. At least 40 genes encoding ICP effective against Lepidopteran, Dipteran, and Coleopteran insects have been isolated and

their DNA sequences compared (Höfte and Whiteley 1989). Purified preparations of 1CPs encoded by cryIA(b) and cryIA(c) genes have been shown to be very toxic against codling moth (Vail et al. 1991).

The cryIA(c) gene was introduced initially using the binary plasmid pWB139 containing the active fragment encoding the insecticidal activity of the cryIA(c) gene from the HD-73 strain of *B. thuriengiensis* (Dandekar et al. 1992). Low levels of expression were observed among 27 independent transformed lines which were the first field introduction of transgenic apple. A synthetic gene was subsequently introduced that had a codon bias compatible with dicots, expressed at levels sufficient to kill all the feeding codling moth larvae (Dandekar, personal communication) and over 400 transgenic plants are under test.

Transgenic 'M.7' apple rootstocks have been obtained expressing *attacin E* gene from the giant silk moth, *Hyalophoras cecropia* (Norelli et al. 1994). Greenhouse and field results indicate that one transgenic line has increased resistance to *Erwinia amylovora* the incitant of fire blight. Attacin E is one of a class of proteins referred to as lytic peptides effective against bacterial infections (Bowman and Hultmark 1987). However, these proteins are rapidly turned over in plants and accumulation to high levels may be required to increase their efficacy. Field trials are in progress to evaluate 'MX and 'Royal Gala' transgenics with attacin E and three other lytic proteins (H.E. Aldwinckle, personal communication).

BREEDING SYSTEMS

The majority of cultivated apples are functional diploids ($2n = 34$) and 17 bivalents form at meiosis (Lespinasse et al. 1976; Zhang et al. 1988ab,c). Although most characters of apple that have been studied are polygenically controlled, the characters under single gene control all demonstrate diploid segregation. There have been suggestions (Darlington and Moffett 1930) that they are complex polyploids, partly tetraploid and partly hexaploid, with the basic number of $x = 7$, which is common in Rosaceae. The basic chromosome numbers of 8 and 9 also occur in Rosaceae, and Sax (1931) put forward the theory that all the Pomoideae were allopolyploids derived from a doubled hybrid between two remote ancestral types, one with the basic number of 8 and the other 9. Dermen (1949), while agreeing in general with Sax, considered that, because of the great diversity between genera in the Pomoideae, each genus must have arisen in this way independently. Electrophoretic studies of the inheritance of seven pollen enzymes in apple have indicated bigenic disomic inheritance for five enzymes and monogenic control for two enzymes, results compatible with allopolyploidy (Chevreau et al. 1985).

Triploidy occurs in apple cultivars ($2n = 51$). These have arisen naturally from the fertilization of unreduced gametes. Among the cultivated apples, triploids appear to be more common than one would expect, accounting for about 10% of the commonly grown cultivars, yet appearing in populations from diploid parents only at the rate of approximately 0.3%. A number of triploids are of considerable economic importance, such as 'Baldwin', 'Gravenstein', 'Rhode Island Greening',

'Blenheim Orange', 'Stayman Winesap', and more recently 'Jonagold' and 'Mutsu'. In fact, all of the promising seedlings that are assumed to derive from 'Winesap', such as 'Stayman Winesap' and 'Mammoth Black Twig', are triploid. It would appear that there is more chance of a spontaneous triploid seedling being outstanding than a diploid. They are more vigorous and tend to have larger fruits, which might account for some of their selective advantage. Triploids, however, produce very poor pollen and this leads to pollination problems in the orchard since it requires a diploid to pollinate the triploid, and another diploid to ensure fruit on the diploid pollinizer. The triploids produce few viable seeds; nevertheless they are sufficiently fertile to produce a good crop of fruit if properly pollinated. The lower content of seed that they contain (because of aneuploidy) might also contribute to annual bearing. They were considered useless as parents for further breeding since, when selfed or crossed, practically all seedlings would be aneuploid, which are weak and seldom develop into trees. Of 329 seedlings from open-pollinated triploids; 2 (0.6%) were haploid, 6 (1.8%) were diploid, 4 (1.8%) were triploid, and 10 (3.0%) were tetraploid, while 307 (92.8%) were aneuploid (Einset 1945). Although triploids as pollen parents can be expected to produce very few 34 chromosome gametes, the $2x$ gametes being balanced will have a selective advantage over aneuploid gametes. 'Hokuto', a triploid seedling, was selected from 79 seedlings resulting from the cross between diploid 'Fuji' and triploid 'Mutsu' as the pollen parent (H. Bessho, personal communication).

Tetraploids ($2n = 68$) have been discovered as large-fruited sports on trees of diploid cultivars. Usually such tetraploid chimeras are of no commercial value. Shoots are typically thick and sparsely branched with short internodes, and fruits are large, often flattened, and sometimes misshapen. At least two tetraploid forms of 'Yellow Transparent' were significant improvements over the normal diploid cultivar and were planted on a limited commercial scale. The tetraploid giant sport 'Perrine Transparent' produced a semi-dwarf tree carrying regular crops of well-shaped apples, 60 to 65 min in diameter, in contrast to the very vigorous, highly biennial diploid 'Yellow Transparent' with fruit 40- to 50-mm-diameter fruit. Spontaneous tetraploids usually occur as chimeras with one or more layers of diploid tissue over the tetraploid. If L-2 is tetraploid, the giant sport will produce segregating $2x$ gametes and the progeny in crosses with diploids will be triploid. By crossing those that breed as tetraploids with diploids, large progenies of triploids can be produced. However, these are not quite the same nor as valuable as triploids derived from an unreduced diploid egg cell and fertilized by $1x$ pollen.

The explanation for the superiority of these natural triploids given by Knight and Alston (1969) is that in triploids produced from $4x \times 2x$ parents, the gametes from both parents have undergone meiosis and therefore have a random reassortment of genes. Natural triploids, however, usually arise from the fertilization of an unreduced egg cell by a haploid pollen grain. The pollen has undergone full segregation, but the maternal gamete will have either undergone no segregation or a minimum of reassortment and therefore contributes to the resulting triploid two-thirds of the genomic constitution of the maternal parent intact or slightly changed

from a parent that was probably a very successful cultivar. This preserves the essential quality of the desirable maternal parent, which may be enhanced by an additional genome as well as by the triploid condition. 'Jonagold' (a cross of diploid 'Golden Delicious' × diploid 'Jonathan') is a triploid produced from nonreduction in 'Golden Delicious', but it is unclear if the complete genome of 'Golden Delicious' is intact or if some reassortment has occurred, perhaps, from second-division restitution in meiosis.

The problem in taking advantage of nonreduction to produce triploids is that the frequency of their occurrence is low. Thus, many seedlings have to be grown to get a reasonable number of triploid seedlings from which to select. The method used by Knight and Alston (1969) is to save seeds from discarded fruit from a packinghouse where the seed parent is known and perhaps even the pollenizer. By selecting the largest seeds from the mass of seeds saved, growing seedlings, and selecting for leaf size and vigor, a number of triploids can be obtained without growing thousands of seedlings.

Tetraploids can also be produced from triploids pollinated by diploids. The number of good seeds produced from such crosses is very small and most produce aneuploid seedlings. Laubscher and Hurter (1960) made root-tip counts of 884 seedlings from triploid cultivars growing in a mixed orchard of diploids. In all, eight tetraploids were obtained, or one in 110 seedlings. Not all triploids respond in the same way and some produce many more tetraploids than others (Einset 1952).

The tetraploids are presumed to arise from unreduced 51-chromosome egg cells fertilized by 17-chromosome pollen, and it is suggested that tetraploids produced in this way may be better parents for breeding than those from colchicine-treated diploids. The first tetraploid cultivar to have been bred in this way is the Swedish 'Alpha 68'. Hexaploids have also been obtained by colchicine treating triploids; 6-3-3 and 3-6-6 chimeras have been produced of the cultivars 'Paragon' and 'Stayman Winesap' (Dermen 1965). One disadvantage of many of these polyploid seedlings is that they have very long juvenile periods (some of the natural triploids may be an exception) and long juvenility is correlated with slowness to come into bearing after propagation of adult material.

Sterility and Incompatibility

There are two main causes of unfruitfulness in the apple: sterility and incompatibility. Generational sterility can be brought about by the failure of any of the processes concerned with the development of pollen, embryo-sac, embryo, and endosperm. This is evident in triploids where chromosome imbalance in gametes results in only a small percentage of good pollen being produced and poor seed set. Some diploids also have high amounts of aborted pollen, probably due to lethal recessive genes present in the heterozygous condition in the sporophyte.

Sexual incompatibility that is due to the failure of the pollen to grow down the style and bring about fertilization is widespread in apple. Self-incompatibility is particularly common, although cases of cross-incompatibility are also known. Apples

have a gametophytic incompatibility system whereby the pollen tube growth is arrested in the style. Pollen germination is not noticeably lower in incompatible pollinations than in compatible ones and is unrelated to subsequent pollen tube growth. Research on the S allele of apple should aid in understanding the system (Battle et al. 1995; Broothaerts et al. 1995).

Almost all apple cultivars are self-incompatible to some extent, and some are completely so. Even those that appear to be self-compatible set more fruit with higher seed content when pollinated with a cross-compatible cultivar. Brittain (1933) selfed 'Cox's Orange Pippin', 'Golden Russet', and 'Northern Spy' on a large scale-several thousand flowers of each-and the mean fruit set was about 1.5%, compared to 7% from cross,-pollination. Cross-incompatibility is not very common, but a number of cases have been reported, usually between close relatives. Sports of a cultivar are cross-incompatible with the original form and with its other sports, but this is essentially self-incompatibility.

Triploid cultivars behave in much the same way as diploids, varying in the degree of self-incompatibility and producing an increase in fruit set when pollinated with diploids, but varying considerably when crossed with other triploids. Natural tetraploids are also very variable, some being possibly self-fertile and others only partly so because gametophytic incompatibility is typically voided in diploid gametes. In a series of crosses (Brown 1975), the following results were obtained: $2x \times 4x$ gave 9% fruit set; $4x \times 2x$ gave 3% fruit set; $3x \times 4x$ gave 7% fruit set. Other information suggests that $4x \times 4x$ sets fruit freely.

Apomixis

Some plants, while appearing to produce seeds in the normal fashion, actually reproduce from unfertilized eggs, from diploid cells of the nucellus, or from some cell of the megagametophyte. This form of reproduction is known as apomixis. Some plants produce both sexual and apornictic seed. If seeds are produced from the diploid maternal cells of the nucellus, the resulting plants will be identical to the maternal parent and show no segregation. Thus, this type of apomixis is a form of vegetative propagation. If seeds form from haploid eggs, the resulting plants will show segregation and may be haploid or, if doubled naturally or with colchicine, will give rise to a completely homozygous individuals. There are various techniques to produce haploids. Haploids may occur spontaneously (typically one in a thousand seeds), but they are very weak and generally do not survive. They can be selected by pollinating with a homozygous dominant seedling marker and selecting for seedling recessives. Using this technique, 13 haploids were identified (Lespinasse and Chevreau 1987) and some of these haploids have been doubled. Other techniques suggested include (1) in vitro androgenesis. by anther culture, (2) in vitro gynogenesis by unfertilized ovule culture, and (3) in situ parthenogenesis by irradiating pollen followed by in vitro culture of immature embryos (Hofer and Lespinasse 1996). While progress has been made, with these techniques, have not as yet been successful at producing viable haploids in apple.

Homozygous plants of apple represent excellent experimental material for cytological and genetic studies. but the breeding value of haploids has been overstated. A random haploid or doubled haploid has no real value except as a genetic model. Two random double-haploid apples when crossed will produce uniform F_1 progeny but this genotype will probably be no better than a random heterozygous selection obtained from a diploid × diploid cross. There is no evidence to support expectations that natural selection for viable haploids will be related to horticulturally desirable parents, although one might assume some natural selection for vigor since deleterious recessives will be lethal in haploids. Further, it will be much more difficult to select promising hybrids because the chance of selecting two promising haploids will be extremely low and they must be tested by intercrossing, and then their progeny evaluated, an expensive technique. This is a very inefficient way to produce apple selections because superior genotypes produced from heterozygous diploid × heterozygous diploid can be fixed by vegetative propagation. Seedling apples will also be juvenile, which will be a problem. This method might be a promising technique to produce seed-propagated homogeneous rootstocks because they would be free of virus, but it seems unlikely that this would be less expensive that the present stoolbed production of virus-free clones. However, the limitations cited for fruiting cultivars applies also to rootstocks.

Facultative apomixis is characteristic of a number of *Malus* species that are probably of hybrid origin, but does not appear to occur among the cultivated apples. The apomictic species that have been investigated are polyploids. *Malus sikkimensis* is a triploid; *M. coronaria, M. hupehensis, M. lancefolia* Rehd., *M. ×platycarpa,* and *M. toringoides* (Relid.) Hughes are known in both triploid and tetraploid forms; *M. sargentii* is tetraploid, and *M. sieboldii* is known in diploid, triploid, tetraploid, and pentaploid forms. It is probable that the so-called diploid forms of *M. sieboldii* are sexual hybrids with other species.

Under normal circumstances these species reproduce themselves freely by apomictic "seeds" but most of them can produce sexual hybrids if crossed with sexual diploids. Sax (1959) has demonstrated that the tetraploid *M. sargentii* when pollinated with sexual diploids produced maternal tetraploids and hybrid triploids and pentaploids. Seedlings from these apomictic species are not necessarily identical and a certain amount of variation can be found. The importance of this character in *Malus* species is that the seedlings of some are sufficiently uniform to be used as rootstocks that are virus free. There are certain problems of stock-scion incompatibility and sensitivity to virus which will be dealt with in the section on apple rootstock breeding. Hanna Schmidt (1958) has demonstrated that it my be practical to create apomictic parents that will produce clonal rootstocks by seed. Some of her *M. sargentii* × 'M.9' selections are facultative apomicts, producing 60 to 90% maternal clones. These could be identified by using a homozygous red-leafed pollinator and roguing the red seedlings. Orchard evaluations of seedlings from the *M. sargentii* × 'M.9' selections has not been encouraging; yields have been low and fruits small (Schmidt, 1988).

The inheritance of apomixis is not very straightforward. Sax (1959) considered apomixis to be a dominant trait in the F_1 hybrids from controlled crosses, whereas

the evidence from Schmidt (1964, 1988) shows it to be recessive but varying according to the ploidy of the species. Triploid hybrids of apomictic × amphimictic forms of *M. sieboldii* were in the ratio of 6 amphimicts: 1 apomict, but in crosses between high chromosome number apomicts and low chromosome number amphimicts, the proportion of apomicts was higher and increased proportionally to the difference in chromosome number.

Parthenocarpy

The apple usually has 10 ovules but it is not necessary for them all to be fertilized and develop into seeds for a fruit to be produced. Often a single seed is sufficient for the development of the fruit; thus fruitfulness may still be maintained even when a high degree of generational sterility is present. It is even possible in some cultivars and in certain conditions for fruit to develop parthenocarpically without fertilization and without weds. Fruits that arise in this way vary according to the cultivar: In some they are small and often misshapen, and in others they are normal in size and appearance. These fruits tend to ripen earlier and do not keep as well in storage as seeded fruits.

In a classic, elegant experiment using removal of seeded fruit from apetalous cultivars 'Spencer Seedless' and 'Ohio 3', Chan and Cain (1967) demonstrated that it is the presence of seeds inhibits subsequent flower formation and not the nutritional competition of the developing seedless fruit. The critical period was 3 weeks after pollination. The two apetalous cultivars bear annual crops of parthenocarpic seedless fruits (because insects do not visit the apetalous flowers) but produce seeded fruits following hand pollination.

The use of cultivars that produce parthenocarpic fruits consistently has been recommended because of their ability to produce good yields in years when flowers are damaged by late spring frosts or when conditions are unfavorable for pollination (Thiele 1950). Some quite heavy-cropping cultivars have been described that produce parthenocarpic fruit, but none seems to have been grown to any extent. Ewert, as long ago as 1909, was advocating the planting of seedless apples as a safeguard against failure of fertilization. Others have suggested breeding for this character, but until recently little breeding was carried out. The combination of apetaly with columnar apples has been achieved by crossing 'Wellington Bloomless' with 'McIntosh Wijcik' and backcrossing the columnar normal flowered seedlings to the apetalous 'Spencer Seedless' (Tobutt 1994). This combination could be useful to avoid bienniality and to achieve consistent production in high-density orchards without pollination, allowing production to be independent of bee activity.

Inbreeding

Inbreeding in apples has generally been considered to be an unsuitable method of breeding because of the expected loss of vigor. Vigor could be restored by intercrossing inbreds but this is an unpromising breeding approach because

TABLE 3. Relation of Mortality and Vigor With Inbreeding

Inbreeding coefficient	Mortality (%)	Avg. height (m) after 6 years
0.000	3.2	2.62
0.625	29.5	2.00
0.875	55.0	1.56

Source: Data from Brown (1975).

heterozygous individuals obtained by intercrossing two heterozygous parents can be clonally propagated. However, because excess vigor requires expensive annual pruning a slight reduction in vigor induced by inbreeding may be appropriate in some areas.

Any program of inbreeding by selfing is difficult to pursue with apples because of the high degree of self-incompatibility. A great many flowers have to be selfed to produce a few fruits from which only a few weds are obtained. The average fruit set from selfing a great many cultivars is about 2% and the number of seeds per fruit averages one to two. Only about 30% of the seeds germinate and a great many of these are weak and die, probably as a result of lethals becoming homozygous. The end result is that only a very small proportion of the seeds produce viable seedlings capable of flowering and fruiting. In these selfed progenies it is possible to get a few good seedlings which, if not completely self-incompatible, can be selfed again. By selecting the most vigorous and most self-fertile it has been possible to carry this process through three selfed generations at the John Innes Institute, Norwich, England. However, a comparison of seedlings derived from three progenies with different degree of inbreeding of 'Cox's Orange Pippin' indicated increased mortality and decreased vigor as a result of inbreeding (Table 3). Karnatz (1988) reinvestigated selfing as a breeding method and selected some promising selections from inbreeding 'Golden Delicious'.

Inbreeding is increased when parents have common ancestors. Thus, it is important when making crosses between apparently widely different cultivars to consider their pedigrees. Inbreeding may result from intercrossing new cultivars because so many of them are based on 'Golden Delicious' or 'Delicious'.

Outbreeding and Backcrossing

Most breeding programs are based on outcrossing, usually between cultivars of known merit, with the object of combining the good qualities of both parents in some of the progeny. Much of the present breeding is aimed at specific objectives, and complementary parents are selected that are expected to impart some specific character to the offspring. In such cases a certain amount of backcrossing is used to introduce one specific character into another background as in single gene resistance to disease. It must be remembered that inbreeding occurs at the same rate in self-mg and backcrossing.

Dominant single gene resistance (as *Vf* gene for scab resistance) in Malus species has been transferred to the cultivated apple by a modified backcross procedure to avoid inbreeding (Crosby et al. 1992). The method involves crossing the wild species (heterozygous for VJ) with a large-fruited cultivar. The resistant Fig are heterozygous and the best are selected and backcrossed to a good cultivar. Their progeny yields 50% seedlings carrying the dominant gene identified by scab resistance. The best of these are again backcrossed to suitable cultivars and so on until all the suitable qualities of the cultivated apple are recovered and the resistance from the species is retained. Inbreeding is avoided by alternating different cultivars for the "recurrent" quality parent, which eliminates loss of vigor and incompatibility problems.

Characters Controlled by Single Genes

In the early days of genetics, when the simple segregations from single gene differences were being discovered, it was thought that plant breeding would be revolutionized, and so it was for some plants. But when fruit trees were crossed the results did not fit any of the simple segregations and no single gene characters were known for a long time. All the important characters, such as fruit shape, size, and color, were shown to be inherited quantitatively. In those days this was not easy to understand, and Mendelian genetics was thought to have little to offer the fruit breeder. Since then a number of single gene characters have been found, particularly in relation to disease resistance, but also other characters such as albinism, apetaly, lethals, and columnar growth (Table 4).

TABLE 4. Genes of the Apple

Preferred symbol[a]	Original symbol	Gene Effect	Source	Reference
		Pest resistance		
Er		*Eriosoma* resistance	'Northern Spy'	Knight et al. (1962)
Sd_1		*Dysaphis devecta* resistance	'Cox's Orange Pippin'	Alston & Briggs (1968)
Sd_2		*Dysaphis devecta* resistance	'Northern Spy'	Alston (1970b)
Sd_3		*Dysaphis devecta* resistance	*M. ×robusta* MAL 59/9	Alston (1970)b
Sd_{pr}		*Dysaphis devecta* resistance	'McIntosh'	Alston & Briggs (1977)
Sm_h		*Dysaphis plantaginea* hypersensitivity	*M. robusta* MAL 59/9	Alston & Briggs (1970)

TABLE 4. (Continued)

Preferred symbol[a]	Original symbol	Gene Effect	Source	Reference
		Disease resistance		
G_b		*Glomerella cingulata* susceptibility	'Golden Delicious'	Thompson & Taylor (1971)
$G_{y\text{-}a}$ $G_{y\text{-}b}$		*Gymnosporangium* resistance	'Spartan'	Aldwinckle et al. (1977)
P_c		*Phytophthora cactorum* resistance	'Northern Spy'	Alston (1970b)
Pl_1		*Podosphaera leucotricha* resistance	*M. ×robusta* MAL 59/9	Knight & Alston (1968)
Pl_2		*Podosphaera leucotricha* resistance	*M. ×zumi* MAL 68/5	Knight & Alston (1968)
Ps_1		*Phyllosticta solitaria* susceptibility	'Jonathan'	Mowry & Dayton (1964)
Ps_2			'Idared'	Mowry & Dayton (1964)
V_b		*Venturia inaequalis* resistance	*M. Hansen's* baccata 2	Dayton & Williams (1968); Williams & Kuc (1969)
V_{bj}		*Venturia inaequalis* resistance	*M. baccata jackii* Dg27Tl	Dayton & Williams (1968)
V_f		*Venturia inaequalis* resistance	*M. floribunda* 821	Dayton & Williams (1968)
V_m		*Venturia inaequalis* resistance	*M. micromalus* 245-38	Dayton & Williams (1968)
V_r		*Venturia inaequalis* resistance	Russian sdlg. R12740-7A	Dayton & Williams (1968)
		Fruit attributes		
Bp_1Bp_2		bitter pit resistance	Coop 11	Korban & Swiader (1984)
Ca_aCa_b	*AK*	deciduous calyx	*M. zumi*	Henning (1947)
Ma		malic acid content	'Lord Lambourne'	Nybom (1959)
R_f		anthocyanin in fruit skin	'Worcester Pearmain'	Wilcox & Angelo (1936)
R_u		russet	'D'Arcy Spice'	Alston (1973)

TABLE 4. (Continued)

Preferred symbol[a]	Original symbol	Gene Effect	Source	Reference
		Flower or reproductive attributes		
ape		apetaly	'Spencer Seedless'	Tobutt (1994)
at_c		atrophied cololla	'Melrose'	Decourtye (1967)
P_1	a_1	pollen lethal	'Reale d Entraygues'	Heilborn (1935)
P_2	a_2	pollen lethal	'Transparente de Croncels'	Heilborn (1935)
P_3	a_3	pollen lethal	'Germaine (loire)'	Heilborn (1935)
P_4	d_1	pollen lethal	'Carrey'	Heilborn (1935)
P_5	d_2	pollen lethal	'Cellini'	Heilborn (1935)
P_d		double petals	'Dorothea'	Sampson & Cameron (1965)
S_1S_2		incompatibility (multiple alleles)	'Northern Spy'	Knight et al. (1962)
		Plant attributes		
al	C_1	albinism	'Rev. W. Wilks'	Hall & Crane (1933); Crane & Lawrence (1933)
bu_1bu_2	*tu*	burrknots	'Northern Spy'	Decourtye (1967)
Co		compact habit	'McIntosh' (Wijcik)	Lapins & Watkins (1973)
d_1		dwarf	Ottawa 521	Alston (1976)
d_2		dwarf	'Northern Spy'	Decourtye (1967)
d_3		dwarf	Ottawa 521	Alston (1976)
d_4		dwarf	'Starkrimson'	Alston (1976)
G		dwarf re-growth promoter	Ottawa 521	Alston (1976)
l	C_2	pale green lethal chlorophyll deficiency	'Northern Spy' 'Calville Blanc d'Hiver'	Klein et al. (1961) Williams (1958)
n_b		necrotic bark	'Reinette du Mans'	Lespinasse (unpub.)
R_t	*B*	purple pigmentation in all tissues	'Baskatong'	Lewis & Crane (1938); Sampson & Cameron (1965)
S_b		sieboldin present	*M. floribunda* 821	Alston & Hunter (1971)
W		weeping habit	*M. baccata gracilis*	Sampson & Cameron (1965)
$ym_1ym_2ym_3$	$a_1a_2a_3$	yellow mottle	'Worcester Pearmain'	Sadamori et al. (1964)

[a]Indicates change from original symbol in accordance with nomenclature recommendations.
Source: Brown (1992).

One of the first characters to be found in the apple that was controlled by a single dominant gene was the complete anthocyanin pigmentation originating in *Malus pumila niedzwetzkyana* Schneid. (Lewis and Crane 1938). Within the red-leafed seedlings derived from crossing *M. p. niedzwetzkyana* with normal green apples, there is considerable variation in the intensity of the pigmentation, suggesting that other factors am involved. Nevertheless, in this case one can make a positive classification into those that we pigmented and those that are not. Homozygous red occurs rarely. 'Royalty' is one of the few cultivars producing 100% red-pigmented progeny. Most red-pigmented apples produce more non-red progeny than predicted. A red × green cross may yield as few as 15% red-pigmented progeny (J.N. Cummins, unpublished).

Where a character is controlled by a dominant gene, it is very easy to transfer it into other apples. The number of generations required depends entirely on the quality of the apple in which the desired character is found. If it is already within the cultivated apple, then possibly only one generation is required; if, on the other hand, it is in a species of *Malus,* then several generations, possibly five or six, of crossing and backcrossing may be required to eliminate the undesirable characters of the species and retain the good qualities of the large-fruited cultivars and the desired character of the species. However, if we assume even a minimum of 4 years per generation, 5 backcrosses require a minimum of 20 years, a daunting prospect for any but very young breeders. The possibility of genetic transformation could shorten this interval considerably.

When the character is recessive (as *apetalous),* as are most mutations, then the breeding becomes much more complicated and protracted. When a cultivar carrying a recessive character is crossed with a normal, the F1 is normal. The recessive character reappears in a quarter of the F_2 resulting from sib crossing or in half of the backcross to the recessive parent.

Characters Controlled by Polygenes

Fruit size, shape, and productivity are under polygenic: control, which means that when two cultivars are intercrossed there will be a wide and continuous range of expression of all these characters in the seedlings. They will not segregate into discrete categories. Most polygenic: characters behave independently. The range of variation is related to the expression of the characters in the parents and the progeny mean is always related to the parental mean. In some, given a strictly additive system where there is no dominance, the progeny mean and the parental mean will be the same; in others it may be above or below the parental mean. The extent of the deviation and its direction will depend on dominance or epistasis and on the difference between the dominant and recessive phenotype.

When the number of seedlings is plotted against the character values a simple distribution curve is formed about the mean. In cases where no dominance is present and the parental values are known, it is easy to predict the progeny mean and the proportion of the progeny that will be above and below the parental values. Characters

that behave in this way appear to be those that have not been subjected to great selective pressure. Fruit shape is a character where there has been no selection at either end of the scale for particularly flat apples or tall ones. There are cases where there is a limit in the values of expression. Thus if July is considered the earliest that an apple can ripen because this is the minimum time from flowering in which a fruit can develop, then July is a fixed low value. The progeny from two early-ripening cultivars will show a one-sided distribution because of the July barrier and will produce only seedlings that ripen in July and later but none earlier. In such cases the progeny mean will be higher than the parental mean.

It frequently happens that the parental mean is considerably greater than the progeny mean although the distribution forms the same simple curve. This occurs with characters that have been subjected to extreme selective pressure over many generations. Where selection is always at the extreme end of the curve, the progeny mean will tend toward the middle of the range of variation for the character. An example of this is fruit size, where strong selection has been away from the small-fruited crabs and progeny mean nay be as much as 40% less than the parental mean (Brown 1960). However, the breeder should be aware that many characters are distorted by metrical bias. Thus, crossing large and small apples often gives hybrids that seem closer to the small parent suggesting dominance for small size. Plotting fruit size on a log scale will often show that the hybrid is at the midpoint, suggesting that the genes for size work on a logarithmic increase rather than on an arithmetic increase.

In addition to having characters controlled by either a single major gene or by polygenes, it is possible to have one system superimposed on the other. This can present a rather confusing picture. Such a situation exists in the inheritance of malic acid concentration in the fruit where medium to high acid is dominant over a very low acid type, but within both types there is a range of variation typical of polygenic control (Brown and Harvey 1971). In many quantitative characters where many genes are involved, major control is often exerted by a relatively few genes, each with a large effect. These few genes may be identified by linkage with molecular markers. Thus, the mapping of the apple genome could advance selection even for so-called quantitative traits.

Parental Contributions

Where detailed fruit and tree records of seedlings of many progenies have accu-mulated over many years, it is possible to use these data to calculate the contribu-tions made by individual parents to their progenies. This assumes that the data for the different characters have been recorded in such a way that it is possible to clas-sify the seedlings into categories suitable for analysis. Gilbert (1967) describes a method of analyzing a series of biparental crosses that have some parents in com-mon by which the main effects of each parent on each of the characters can be calculated by treating the data as an incomplete diallel cross. Since the combining abilities are additive, the calculated main effects for each parent allow predictions

to be made with considerable accuracy as to the progeny mean of untested combinations of parents.

Selection of Parents

Since most apple breeding is based on selection from progenies produced by crossing extremely heterozgyous parents, consideration must be given to parent selection; otherwise, breeding becomes haphazard and the outcome unpredictable. If the major aim is to introduce a character that is controlled by a major gene or genes, then the choice of at least one parent is limited to cultivars carrying these genes. However, most of the important characters are polygenically controlled; for each there will be a considerable range of variation and the breeder seeks the optimum expression of each. Fortunately, the breeding behavior of highly heterozygous parental lines, with respect to measurable characters having a low level of genetic dominance, is often predictable. As has been shown, the mean value for the progenies falls with reasonable consistency around the mean of the two parents. It should be the aim to try to combine parents that between them have all the chosen characters present at as near the optimum expression as possible. Thus if polygenic mildew resistance is desired, then one and preferably both parents should have a high degree of resistance in order that as many resistant seedlings as possible are available from which to select for other characters. It is probably more realistic to choose parents with complementary characters in order to select progeny that have the desirable attributes of each parent.

Having chosen the parents, the breeder must determine the size of the progeny required to be reasonably certain of obtaining a new apple having all the attributes visualized when planning the cross. Using actual records, Williams (1959) calculated that the percentage of desirable seedlings that can be expected as the main product of an apple breeding program for polygenically controlled characters is seldom more than 40% and for every additional character the figure rapidly decreases. Thus for a program in which the main objective is polygenically controlled mildew resistance, size of fruit, season of maturity flavor, and color of skin, a reasonable estimate would be 40, 20, 20, 10, and 10%, respectively. Assuming that these characters behave independently, a progeny of 6250 (16/100,000) would, on the average, yield one seedling possessing a combination of the five traits at an acceptable level of expression; the progeny size would have to be even larger to expect the rare recombinant to occur with a high probability of success. However, experience with rootstock screening indicates that these estimates may be overly optimistic by an order of magnitude (Cummins and Aldwinckle 1983). In addition to these definable characters there are others for which allowance must be made, as well as some that cannot be readily included in the calculation, such as minor characters of flavor or texture which so often decide the ultimate success or failure of a selection. It is not unreasonable to assume that an actual population as high as 30,000 will be required, and to observe 30,000 seedlings would be practically impossible if the seedlings are

to be evaluated at the fruiting stage. Nevertheless, in any serious breeding program, provided that great care is taken in the selection of the parents, progenies as large as practicable should be aimed at. Instead of growing all the seedlings to fruiting, as many as possible should be eliminated in the early stages of growth. For example, a high proportion might be eliminated because of an unacceptable level of mildew susceptibility by the second season of growth. Results, however, can be improved by increasing the number of cycles of selection.

Breeding Strategy

Bringhurst (1983) contributed a penetrating analysis of fruit breeding based on his experiences as a breeder of strawberry and avocado. His conclusions seem particularly appropriate to apple breeding where efforts have only recently been more effective than evaluation of naturally occurring variation.

The traditional strategy for fruit breeding has been to identify superior phenotypes, propagate the best selections, develop cultural practices that enhance the performance of the selected cultivars, hybridize among the best selections, and then continue the cycle. This breeding method may be considered a form of recurrent mass selection in which the key concept is selection of the best individuals and continual recombination over many cycles. The most productive breeders carry on a number of objectives simultaneously and keep the program moving on with the best selections becoming parents for the next generation. The assumption is made that as the population improves, the chance of releasing superior genotypes will increase. Results from a number of crops including apple indicate that this strategy will succeed. Why then did most apple breeding programs fail to make an impact on the industry? Bringhurst's analysis includes the following reasons:

1. *Insufficient support.* Because of the tremendous selection pressure that has resulted in our present cultivars it is extremely unlikely that casual programs can be expected to produce superior. seedlings. This is borne out by the evidence. Many programs throughout the United States have not been successful and have been dropped; only long-term program have been successful. This require continuous support and a long-term strategy. With deficiency in funding, short-term breeding objectives my be overemphasized to the detriment of long-term objectives. Progress in fruit breeding tends to be incremental and there is no quick Jim.

2 *Faulty strategy.* Genetic progress in recurrent selection requires continuous generations of progress. Attempting to spend most resources on making many crosses to find the best combinations and then repeating appropriate crosses with very large progenies is usually an inefficient use of time and resources. Errors are very costly. Intense examination of unsuitable crosses is folly. No end of testing will compensate for a dearth of good material from which to select.

3. *Misdirected emphasis.* Undue emphasis on secondary traits is misdirected; the breeder needs to emphasize the most essential traits. For example, breeding that concentrates on diseases and insects that can be controlled or we of minor importance is inefficient. The power of the cooperative apple breeding program of Purdue, Rutgers, and Illinois was in its unceasing, single-minded emphasis on moving the *Vf* gene into adapted types.

Other errors include faulty procedures, failure to measure things properly, failure to start at the most advanced level, failure to move through the generations, failure to eliminate the losers (breeders must be ruthless), and failure in breadth of program. Because a breeder of a tree crop such as apple has time for but a few generations, several programs must proceed simultaneously. Finally, Bringhurst points out that every cultivar has its own peculiar characteristics and may require special cultural practices. Consequently, it is in the best interest to the breeder and grower to consider those cultural practices that enhance each cultivars performance.

BREEDING FOR SPECIFIC CHARACTERS

Vigor

The study of the inheritance of vigor is difficult because several factors are involved. For example, parent cultivars we usually grown on rootstocks that control vigor while seedlings are typically self-rooted. In an early study, Spinks, (1936) concluded that there was no correlation between the size and vigor of the seedlings and that of the parents. Watkins and Spangelo (1970) in a diallel analysis of seedling vigor (expressed as plant height) found that the progeny mean was equal to the mid-parent value in one group while in another set there was considerable divergence of the progeny mean from the mid-parent value. This suggests that several factors influence or contribute to seedling vigor. The chief difficulty arises in trying to assess the vigor of parent cultivars. As a practical matter, differences in seedling vigor can be adjusted by the selection of rootstocks.

The effect of inbreeding and aneuploidy in reducing vigor has already been discussed. Interestingly, triploidy increases vigor while tetraploidy decreases vigor, suggesting that the 3x condition is the most physiologically appropriate for apple.

Tree size control, is one of the most important uses of rootstocks. In England the early programs were to increase vigor but the general trend now in apple production is to produce compact precocious trees for ease of harvest. To compensate for the reduced tree size, planting density has been greatly increased in the modem orchard.

There is no consistent semantic distinction between a clone that is dwarf when grown on its own roots or on a vigorous stock and a clone that has dwarfing propensity when used as a rootstock. The term "compact" has been used to describe a tree of small stature regardless of whether its compactness might be caused by genetics, by

a dwarfing stock, or by summer priming (Cummins and Aldwinckle 1983). In an ungrafted plant, dwarfness is typified in the orchard by a spur-type fruiting cultivar such as 'Oregon Spur Delicious', 'Delcon', and 'Nugget Golden Delicious'. However, these genotypes do not induce dwarfing when used as rootstock or interstem. Typical dwarfing rootstocks such as M.8' 'M.9', 'Bud 9', 'P.22', and 'Mark' are all small low-growing bushes that differ from the compact fruiting cultivars.

The physiological mechanisms of dwarfness and dwarfing are still not clearly understood. A number of correlations however, have been claimed for predicting dwarfing capacity of potential rootstocks. Hutchinson (1967) presented data from studies of 'M.9' progenies that suggest that within a family the most dwarfing and the most invigorating individuals can be identified by simple field observations of vigor of ungrafted plants. Beakbane et al. (1939,1941,1947,1975) reported correlations between dwarfing propensity and various structural features. These include the percentage of live tissue of the root cross section; the bark/wood ratio, the percentage of medullary ray tissue in root transection; and high stomatal density of leaves. Some rootstock breeding programs have employed the bark/wood ratio to reduce populations or to select a reduced number of individuals most likely to be dwarfing. Lockard and Schneider (1981) have proposed that the bark is the key to the dwarfing mechanism and the dwarfing bark reduces translocation of auxin, sugars, and other compounds. In the Cornell, Geneva program, orchard testing of grafted trees has been used for estimation of vigor induction. The growth pattern of the orchard tree appears to be well established by the fifth year of testing.

The relation between rooting of branches and dwarfing has been known since the Middle Ages. While not all apples that root readily by adventitious roots from mature shoots are necessarily dwarfing when used as rootstock, all of the most common dwarfing rootstocks possess this character (Tukey 1964).

Dwarfing capacity appears to be under control of a number of genes (J.N. Cummins, unpublished), but few studies on the subject have been published. In a progeny of 'M.9' × 'M.8' both very dwarfing, about one-quarter of the seedlings were as dwarfing as the parents, but progeny midpoint was much more vigorous than the mid-parental mean. Less than 5% of seedlings from crosses of 'M.9' with vigorous parents have been as dwarfing as 'M.9' and as few as <1% in some crosses. The picture is further complicated by the fact that fruit bearing itself is dwarfing and few studies have examined this direct effect of precocity.

Cold Hardiness

In severe winter climates the most important character of the tree is cold hardiness, for many cultivars are seriously injured and, in extreme cases, even killed. There is a continual need for apples of quality that are more winter hardy so they may escape damage in conditions of severe cold in existing apple growing areas and be extended into colder areas. There are considerable differences in cold hardiness among various cultivars and species and it is quite possible to breed for increased hardiness. The

breeding and selection for cold hardiness in deciduous fruit crops was reviewed by Quamme and Stushnoff (1983) and Saveljev (1988).

To survive freezing temperatures, plants have mechanisms either to avoid or to tolerate ice within their tissues by freezing point depression and supercooling. Woody species such as apple have the ability to maintain supercooled cellular water in critical tissues to –40°C. Deep supercooling involves freezing avoidance to the point of spontaneous nucleation of the cellular solutions. Freezing in deep supercooled tissue is lethal when it occurs and the degree to which deep super-cooling occurs determines the poleward limits of production. Detection of the supercooling point provides the basis for measuring the level of cold hardiness. Tissues of apple, however, can survive to liquid nitrogen temperature (-196°C) when fully acclimated. This technique has been utilized to store shoots cryogenically for germplasm preservation (Sakai 1984).

Several techniques can be used to evaluate winter hardiness. The traditional technique was to rely on test winters, but this is unreliable. Much winter injury can be traced to severe low temperatures in the late fall, that may injure trunk and scaffold branches before cold hardiness develops. Acclimation is triggered by environmental cues, including shortening day length, changes in light quality, and temperature drops. Much freezing damage is due to unseasonably warm weather in the winter that results when deacclimated tissues suffer subsequent freezing. It is important to use objective and quantitative methods of testing cold hardiness because field observation under natural conditions is not always reliable. These include artificial freezing tests using portable chambers where damage is evaluated by tissue browning or one of several objective methods of measuring cell viability. Conductivity tests are based on the fact that shoots or other parts of the plant, after having been frozen and soaked for a certain time in pure water, exude electrolytes by leakage from the killed cells and to a less extent from living cells. The amount of electrolyte exuded may be estimated by measuring the electrolytic conductivity of the water. Exotherm analysis detects the heat of fusion by a deflection on the time-temperature profile (exotherm) during constant cooling. This approach has been used to determine cultivar differences in wood hardiness of apple.

Inheritance of cold hardiness is under polygenic control with the frequency distribution in progenies forming a normal curve about the mean. In most progenies there air some seedlings more hardy than either parent. Watkins and Spangelo (1970), using a diallel analysis of seedling apples derived from parents that were unusually hardy and evaluated with artificial freezing, demonstrated that additivity was a major component of genetic variance for winter survival.

Tolerance of low winter temperatures has been the central objective of numerous breeders across Canada, the northern United States, Sweden, Poland, northern China, and Russia (Saveljev 1988). These hardiness-centered programs have generally been intended to extend the range of apple production for local consumption, rather than to develop cultivars to compete on the broad commercial market. Half a century ago, the hardy cultivars available for marginal areas included 'Duchess of Oldenburg', 'Yellow Transparent', 'Wealthy', 'Charlamoff, and several

'Antonovka' types, all of relatively low quality but capable of surviving, even thriving, through the challenges of a severe winter. Breeders of winterhardy cultivars have been especially successful; introductions include 'Fantazia', 'Fireside', 'Handson', 'Honcycrisp', 'Lobo', 'Kaljo', and 'Mantet', 'Northern Lights'. Winter-hardy rootstocks derived from. northern breeding programs include 'Alnarp 2', 'Bemali', the Budagovski series from Russia, 'Mark', and the P-series from Poland.

Winter Chilling Requirements

Apple trees require a certain amount of chilling during the dormant season for their proper development. In subtropical climates this requirement is not always met and prolonged dormancy or delayed foliation results. Cultivars with low chilling requirements occur locally but most are of poor quality. Oppenheimer and Slor (1968) in Israel crossed some of these with good quality cultivars and introduced 'Anna', 'Ein Shemer', and 'Schlor', cultivars with low chilling requirements and much improved quality. Early leafing was the criterion for selection; seedlings that broke bud within three weeks of the earliest were grown. This character appears to be polygenically controlled; about 30% of the F_1 progeny and 40% of the back-crosses to 'Delicious' and 'Jonathan' were sufficiently early to be retained for further testing. Recent discoveries in Mexico and South Africa of 'Golden Delicious' sports with low-chilling requirements suggest other, perhaps simpler mechanisms (Gonzalez-Cepeda 1992). It might be possible to eliminate the chilling requirement since some of the earliest to leaf were earlier after warm winters than after cooler ones. Breeders have utilized germplasm introduced by Oppenheimer 40 years ago to produce cultivars with minimal chilling requirements and with higher quality and better production potential. Low chilling requirement of 'Anna' was postulated to be controlled by one major dominant gene and several minor genes (Hauagge and Cummins 1991). 'Mollie's Delicious' has been used successfully as a source of low chilling in the Brazilian breeding program (F. Denardi, personal communication) Recent low-chill cultivars that are beginning to make an impact on the market include 'Adina', 'EarliDel', 'Goldina', 'Princessa' and 'SummerDel'. 'Primicia' is a new scab-resistant, low-chill cultivar released in Brazil.

Season of Flowering

Since cross-pollination is essential for maximum fruit production in the apple, synchronization of flowering is important. Cultivars that flower very early or very late require special attention to provide efficient pollinators, but this problem has been alleviated by using a range of crabapple pollinizers. It is convenient if new cultivars flower midseason or late midseason for pollination to coincide with that of most of the widely grown cultivars. Late flowering is considered important to avoid disastrous spring frost damage. One objective in a number of breeding programs is to produce cultivars that flower so late that practically all danger to the blossoms from late frost is past. However, progress has been hampered because combining

late flowering with early ripening because of die minimum time required from flowering to the development of full fruit size and maturity.

The inheritance of time of flowering is polygenically controlled. A fair estimate of mean flowering date of the progeny can be obtained from the mid-flowering time of the parents. The seedling distribution about the mean appears to be normal and the spread is such that seedlings flowering later than either parent will normally be found.

Good correlation between the time of leaf emergence and season of flowering was found by Tydeman (1964) and Murawski (1967). Thus, early or even midseason leafing seedlings could be discarded in their second year of growth.

Another contributory factor to crop losses due to late frost is the inherent susceptibility of the flowers to injury. In general, flowers are most tender in the later stages of development, for the styles are most susceptible to damage. Fortunately, not a the flower buds on a tree are at the same stage of development at one time, so that some may escape damage while others are killed. Although there are differences in sensitivity of blossom to frost damage, selection for this character does not appear to have been carried out. Tetraploids and triploids such as 'Jonagold' and 'Mutsu', with larger cells, are more prone to damage than diploids. The hardiness of the tree to winter injury and the hardiness of the flowers to spring frost damage appear to be inherited independently.

Duration of the Juvenile Period

The length of the juvenile period of the seedlings is of prime importance because it determines the number of years the trees have to be grown and occupy space before the results can be assessed. Furthermore, the length of the juvenile period is correlated with fruiting precocity and perhaps with productivity after propagation. Some parents contribute to very short juvenile periods with many of their seedlings flowering in three or four years from seeding, while 'Northern Spy' progenies may take ten or more years to fruit.

The inheritance of juvenility among cultivars is difficult to determine because the length of the juvenile period of old cultivars is usually unknown. Studies of various progenies indicate that the character is quantitatively inherited. Visser (1970) demonstrated a good correlation between the vegetative phase of a cultivar (i.e., the time from propagation to fruiting) and the contribution it makes to the length of the juvenile period of its progeny. Evidence that seedlings must attain a certain size before they reach the stage at which they can flower and fruit would suggest that vigorous seedlings are the ones most likely to attain the optimum size in the shortest time. In general, polyploids have much longer juvenile periods and their seedlings, although they my attain much greater size than diploids, nay take several more years to fruit. Recurrent selection for early flowering can be obtained naturally by using early-flowering seedlings in segregating progeny as parents and by continually discarding all seedlings that do not flower by the fifth or sixth year from planting.

The ability to induce precocity is an important character in rootstock selection. In Cornell, Geneva, this is carried out by using 'Delicious' and 'Northern Spy', notoriously nonprecocious, as test scions on rootstock candidates.

Spur Types

The spur types are characterized by compact habit, reduced internode length, limited side branching on shoots, and prolific development of fruit spurs, producing trees that are precocious in bearing and crop more heavily and regularly in the early years. The spur types are sometimes referred to as dwarfs or compact mutants, but it is possible to have compact trees that do not develop spurs freely. The spur bearing habit occurs as bud sports. Compact strains of 'Delicious' and other cultivars have dramatically changed the apple industry by increasing overall orchard efficiency and permitting use of more vigorous rootstocks. The inheritance of plant form appears to be under polygenic control but it has been difficult to breed for the spur habit in an efficient way because of continuous variation from trees with no or few spurs to trees with many spurs (Lapins 1974; Blazek 1983). Although genetic dwarfs appear superficially similar to compact types, not all dwarfs are spur types. The dwarf character analyzed by Decourtye (1967) was controlled by a single recessive gene and various cultivars, including 'Golden Delicious', are heterozygous. The dwarfs analyzed by Alston (1976) were due to recessives in various combinations.

New spur-types can be produced either by inducing mutations in existing cultivars or by breeding. Mutations can be induced by irradiating dormant scions. The optimum X-ray dose for apples appears to be 3 krad. The treated scions are then grafted onto rootstocks, and the selection for compact types can begin during the first season's growth. The selection criteria are that the length/diameter ratio of the shoots be less than normal and that the shoots have shorter internodes. Selection for short internodes alone is unreliable. The trees are cut back into the original graft, the lateral shoots are cut back to induce latent buds to develop, and the selection method is repeated. This process may be repeated in the third year. These compact selections can be further selected for free spurring and precocious fruiting. These methods are described in detail by Visser et al. (1971).

Not all spur types that have been used in breeding behave in the same way. Lapins (1969) reported that a radiation-induced spur mutant of 'McIntosh' crossed with 'Golden Delicious' did not produce any compact seedlings, suggesting that some of the mutants are chimeras and not necessarily found in the L-2 layer. In contrast, 44% of progeny of the spontaneous compact mutant 'McIntosh Wijcik' when crossed with 'Golden Delicious' had compact growth. Ibis extremely compact mutant is characterized by very short internodes and reduced lateral shoot grown and increased spurriness (Kelsey and Brown 1992). Segregation data indicate that genotype is heterozygous for a dominant mutant named *Co* for *columnar,* but modifier genes influence the segregation ratios and forms obtained. In England, *Co* has been used to produce columnar trees for the home garden, including an ornamental

('Maypole') and a number of dessert types (Tobutt 1985). The Co gene has been combined with *apetalous* in an attempt to produce parthenocarpic columnar-type trees (Tobutt 1994). It has been suggested that the development of apetalous, parthenocarpic, columnar trees with collar rot resistance would permit the use of inexpensive self-rooted trees produced by tissue culture for very high density orchards. This could dramatically change the way apples are grown if problems of excessive vigor can be solved.

In some instances, mutation for spurriness has been associated with delayed ripening of fruit and/or increase in tendency to russet. Several spur-type sports of 'Golden Delicious', for example, ripen 4 to 7 days later than normal 'Golden Delicious', and are much more likely to be russetted. Interestingly, these spur-type 'Golden Delicious' frequently revert to the normal growth habit but retain the delayed ripening and russetting attributes.

Fruit Size

Fruit size is one of the most critical characters in the selection of apple seedlings; if fruits cannot attain the required size, the seedling should be eliminated from further consideration. Fruit size is a somewhat variable character that can be influenced by environmental conditions, and especially by fruit load. Thus, some defects in fruit size can be adjusted by fruit thinning. If seedlings and parents are grown under comparable conditions and fruit load there should be little difficulty in establishing the standard.

There are a number of ways of expressing fruit size; the most common way is diameter. The generally accepted minimum diameter is about 65 mm. In analyzing the inheritance of size, the unit used by Brown (1960) was estimated volume (mL = [(height(mm) $\times$ radius (mm)^2)/1000] $\times$ 2.7. While not as accurate as measurement by displacement, this method permits the use of records of fruit outlines. Classifying seedlings into size groups indicated fruit size to be polygenically controlled with normal distribution about the mean.

The contribution made by parents to size is not quite as straightforward as some other characters. In some progenies a small percentage of the seedlings have fruits larger than the larger parent and quite frequently 50% or more of the seedlings have fruit smaller than the smaller parent. The parental contribution to size can be calculated if data are available from a large number of progenies, by treating them as an incomplete diallel using the method described by Gilbert (1967). This can be much more accurate than trying to estimate the progeny mean from the parental mean.

Fruit size in the apple has been greatly increased by rigorous selection over the years from the small wild types to the size of the present-day commercial apple. Where extreme selection pressure of this kind occurs there is a tendency for the progeny mean to be smaller than the parental mean. This is home out by the analyses of many crosses where the mean fruit size of the progenies is smaller than the average size of the parents by about 34%. This means that many cultivars that are acceptable themselves produce progenies in which the fruit size of the majority of

the seedlings is below commercial size. Some combinations of cultivars are unlikely to yield any or very few seedlings with fruit of acceptable size. In general, the larger the fruits of the parents, the greater will be the proportion of the seedlings producing fruit of acceptable size. It may well be sound policy to select very large-fruited seedlings, not necessarily acceptable as cultivars, for use as parents to ensure an increase in the proportion of seedlings with good fruit size from which selection for other desirable characters can be made.

Fruit Shape

Fruits of apple vary in shape from very flat, oblate to oblong. If the shape is translated into height/diameter and expressed as a percentage, then the very flat apples are about 65% and the very tall ones about 100%. It is rare indeed for the height to exceed the diameter. This procedure does not take into account those conic fruits that taper or other irregularities of contour such as ribbing. Wilcox and Angelo (1937) demonstrated that the tapering conical character is rarely observed in oblate apples (1%) and is most common in the round oblong (32%). By dividing the range of ratio percentages from 65 to 100 into 5% intervals eight shape classes are formed, and by classifying the fruits of seedlings into these categories, it is possible to plot the frequency distribution among the eight classes for the different progenies. The majority of the seedlings fall between the parental values, although the distribution frequency extends beyond the parents and forms a normal distribution curve about the mean (Brown 1960).

By studying a fairly large number of progenies it is possible to calculate the parental contribution to shape of the different cultivars used. In 35 progenies there was a remarkable similarity between the actual progeny mean, the estimated progeny mean (calculated from the sum of the calculated main effects of the parents), and the mid-parent value (Brown 1975). It is possible to use the height/ diameter ratio of the fruit of the parents to predict with considerable accuracy the progeny mean and the range of shape classes expected. Because of the market preference against oblate apples, the breeder will do well to eliminate all oblate apples from consideration as parents.

The amount of ribbing of seedling fruits in a number of small progenies suggests that the progenies from angular-fruited parents will have a preponderance of angular-fruited seedlings (Spinks 1936). Practically all apple progenies will have seedlings with sonic angular, slightly angular, and nonangular fruit, but the proportion in each category differs based on of the parents.

Season of Ripening

Season of ripening is an important character, although transhemispheric shipment and improvement in storage have affected the market demand for very early and very late apples in some areas. However, improved early-ripening and late-ripening cultivars are sought in most countries. A number of studies (Brown 1960; Tancred et al. 1995) indicate that time of ripening is a highly heritable, polygenic character

with predominately additive genetic components of variance responsible for genetic variation. Despite the existence of some specific combining ability and some nonnormal family distributions, the best strategy to predict harvest date is to calculate nz-an harvest date of parents. The progenies from crosses between early-ripening parents have a small range of distribution, which is no doubt due to the limit of earliness being fixed by the minimum time from flowering to fully developed fruit. Thus when very early ripening parents are crossed, the progeny mean is quite close to the parental mean. However, because most very early apples have poor quality, crossing early × early may not be the best strategy; it may be preferable to cross early × high-quality midseason apples and select transgressive, high-quality early segregates.

Breeding for the very hot, dry, and long summer climate of Western Australia that is followed by gradual cooling of night temperatures in late autumn has been successfully carried out by John Cripps. In this challenging climate, even the late-ripening Granny Smith (170 days from flowering) matured during the hot period and quality was unacceptable. Selections of 'Golden Delicious' × 'Lady Williams' delayed the harvest date 2 to 8 weeks after 'Granny Smith'; 'Pink Lady', 'Sundowner', and 'Big Time' mature 2, 4, and 6 weeks after 'Granny Smith', respectively, after the hot season when cooler nights bring good sugar/acid balance and attractive finish. These cultivars hold special promise for regions with similar climates, such as South Africa, the southeastern United States and California, and perhaps Egypt and elsewhere in the Middle East. 'Pink Lady' is now being widely planted in southern France and Washington State.

Fruit Color

Fruit color is determined primarily by the ground color of the skin and secondly by the superimposed anthocyanin pigmentation. The ground color of the immature fruit is dark green. As the fruit matures, (1) the green may fade until it has completely disappeared and the ground color of the fruit will then be in the range from very pale cream to deep yellow; (2) the green may fade but not completely, producing ground colors in the greenish-yellow to yellowish-green range; or (3) the green may not fade at all, leaving a green ground color. The ground color is polygenically controlled and the yellow range (which is related, at least to some extent, to flesh color) and the green range may be controlled independently (Brown 1975).

Anthocyanin production in the fruit skin may be present or absent. If absent, then the fruit is either all yellow or all green. If pigmentation is present, it can take several forms from small red flecks to bold stripes and from a faint blush to solid red. The intensity of the color can vary from very pale to very deep red and the area can be from practically nil to complete coverage. A study of all aspects of fruit color is complex and often confusing because the expression of all these characters can be affected by the stage of maturity of the fruit, by the general environment, by nutritional and cultural factors, and by the microenvironment within the area of the tree.

The production of anthocyanin is normally dominant over the lack of it and the majority of red apples appear to be heterozygous since most when intercrossed produce a few nonred seedlings (Crane 1953). The coloring most often takes the form of striping, but blushes may also occur. These two characters are distinct and these can be stripes, stripes on a blush, or blush alone. Wilcox and Angelo (1936) found that by classifying seedlings into striped and nonstriped (which included the blushed and unpigmented fruit), striping behaved more or less as a single maJor gene producing either 100% striped, 1:1, or 3:1 segregations of striped and non-striped. The blush may be pronounced but in some cases is ephemeral, being extremely light-sensitive. Quite a number of normally yellow apples will, if exposed to full sun, develop a slight blush and it is possible for a few presumed yellow cultivars to produce a few seedlings with colored fruit. Normally when blushed apples are intercrossed, the offspring will be either blushed or nonpigmented but not striped (Spinks 1936).

The shade of red that develops depends to a very great extent on the ground color. The most brilliant red is produced when the ground color is almost white and the dullest brown when the ground color is green. Because dull, dark colors are considered unattractive, ground color may be the most important factor in producing attractive colored fruit. The area of the fruit covered and the color intensity are inherited quantitatively. In most families there is a continuous gradation in color and the average amount in the progeny is proportional to the color of the two parents.

The anthocyanin pigment is in solution in cells of the epidermis and subepidermal layers, but not all cells are pigmented. Some apples have no pigment in the epidermis and the intensity of color depends on the proportion of cells in each subepidermal layer that contain pigment (Dayton 1959). Since overall red is a very desirable character, red sports from many cultivars have been selected that have either greater coverage or more intense red. Most of these sports have arisen naturally but some have been induced by irradiation. It is natural when breeding red apples to select the red sports as parents in preference to the original cultivar, believing the red sports to be better parents. Bergendal (1970) crossed 'Golden Delicious' with six cultivars and their red sports and grew a progeny from each. In all cases the proportion of seedlings in each of the ground color classes was the same from original and sport. In the amount of red, the results from four of the pairs showed no difference, whereas in two the results were quite different.

Misic and Tesovic (1970) examined the pigmentation of the three outer-cell layers of the fruit and showed that the number of pigmented cells in the epidermis of 'Delicious' and 'Richared' are the same, but that in the subepidermis (L-2) there are twice as many pigmented cells in 'Richared' as in 'Delicious', confirming the results of Dayton (1959). The mutation must have taken place in L-2. This indicates that a histological examination of the three outer-cell layers of the fruit skin of cultivars and their red sports would indicate whether a red sport will produce more red-fruited seedlings or will breed the same as the original.

In the past, color sports were extremely important because of a quirk in the patent law. The discoverer of the mutant could patent the new sport and owe no

royalties to the originator of the original cultivar. Thus color sports became a legal way to overcome patent protection. Many licensors of apples now write in the contract that all sports belong to the originator. It is clear that some joint, equitable arrangements need to be made between the originator of the cultivar and the discoverer of improved mutations.

Studies are underway to separate the chimeral nature of 'Gala' sports at the University of Illinois (R. Skirvin, personal communication). Preliminary evidence is that trees fruiting from a red sport of 'Gala' regenerated from leaves produced fruit that were extremely uniform and tended to be poorly colored, suggesting that this technique my not be promising for chimeral engineering. Regeneration from epidermal strips (Compton and Veilleux. 1992) may be a more promising technique.

Russet

Russet on the fruit varies from complete to free with all gradations in between. Complete russet is popular in some countries because it is associated with increased aromatic flavor. Many genotypes have russet confined to the stalk cavity or to the calyx, and others have it in patches over the fruit. Russetting tendencies are inherited but external factors such as low temperature, high humidity during early development, and spray damage can also influence russet production. 'Golden Delicious' is particularly susceptible to russet and russet-reduced sports have been identified. Patchy russet is considered a defect and disliked on apples like 'Golden Delicious', but tolerated on 'Cox's Orange Pippin'.

Information on the inheritance of russet is available from small progenies where russetting was classified as none, slight (russet in the stalk cavity or calyx only), moderate (in patches over the fruit), and complete (Brown 1975). In two progenies where two completely russeted cultivars were crossed, approximately 50% of the seedlings were completely russet and the others slight to moderate. Similarly, when 'Cox's Orange Pippin', a moderate russet, was crossed with the completely russet 'Egremont Russet', 50% of the seedlings had completely russet fruit. 'McIntosh', when crossed with 'Egremont Russet' produced no completely russet seedlings in a rather small progeny. 'Cox's Orange Pippin' crossed with the completely russet 'Golden Russet' produced only 25% completely russet seedlings. In a number of progenies where moderate russets were crossed with slightly russet parents, a few completely russet seedlings where produced. It is obvious from these results that more than one factor is involved since all full russets do not behave in the same way.

Flesh Color

The flesh of the apple varies in color from white through cream to pale yellow; it may be greenish white or it may even be tinged with red. These categories are not discrete; one merges with another. The preferred color is clear yellow because this is accepted by all consumers of the fresh fruit and preferred by processors of sauce and slices. White flesh is accepted and even preferred in regions where 'McIntosh'

TABLE 5. Approximate Percentage Contribution Parental Types to Flesh Color in Seedlings.

Parental phenotype	Seedling phenotype			
	Yellow	Cream	White	Green
White	0	24	22	4
Cream	8	29	8	5
Yellow	35	12	0	3
Green	0	31	0	19

Source: Data from Brown

is considered the standard of apple quality. Red flesh and even very slight bleeding from the skin, are unacceptable.

Brown (1975) has developed a protocol to predict the expected behavior of parents with respect to color. Adding the two parental contributions shown in Table 5 gives an estimate of the distribution of seedlings expected in each color class (although the value for green flesh is based on too few seedlings to be accurate).

Flesh Oxidation

Even more important than flesh color is flesh browning when exposed to air due to the presence of polyphenyl oxidases. Apples that brown are quite unattractive in salads or when served peeled. In the United States almost all producers of apple sauce and apple slices depend on "sulfite" baths of the peeled apple to prevent browning. It now seems probable that this treatment may become illegal, in which case the process may have to confine processing to nonbrowning cultivars such as 'GoldRush' or NY 674.

Inhibition of flesh browning in apples, while important for the fresh market, can be critical for processing. Mechanisms of resistance vary. Cultivars with resistance to browning may have low levels of polyphenol oxidase, low levels of tannins, high levels of ascorbic acid, or some combination of the above. There are no published reports on the inheritance of flesh browning but this trait is under investigation at Cornell University, Geneva. An advanced selection, NY 674, with low levels of polyphenol oxidase and excellent resistance to browning has been crossed with a range of cultivars varying in susceptibility; an analysis of their progenies should separate the components of flesh browning and their inheritance.

Flesh Texture

Flesh texture is increasing in importance in fruit acceptability but there has been little published work on the evaluation or on the inheritance of this character. This is a complex character and there appears to be a relationship between flesh texture and ethylene production. Cultivars differ widely in flesh texture and the persistence

of this character in storage. 'McIntosh' is considered unacceptably soft fleshed, while two new cultivars, 'Honeycrisp' and 'GoldRush' have remarkable crispness. The crispness of 'GoldRush', persists for a year in refrigerated air storage.

Fruit Flavor

Fruit flavor, one of the most important criteria in selection of apple seedlings, is difficult to analyze since the constituents of flavor are a complex combination of acids, sugars, tannin , and aromatic substances. A review of apple flavors by Yahia (1994) indicated the presence of literally hundreds of volatiles but the precise role played by individual components or by various combinations in flavor is not understood. Although the appreciation of flavor is personal and variable (*De gustibus non disputandum est*), the marketplace has made a decision on acceptability.

The basis of apple taste and flavor is acidity and sweetness; it is the balance between these, irrespective of aroma, that primarily determines the acceptability of the fruit. Apples that are high in acid and low in sugar are quite unpalatable, being too acid; similarly, apples high in sugar and low in acid are too sweet and insipid. The acid in the mature fruit is almost entirely malic acid, and is measured either as percentage of malic acid in the fruit juice or as the pH of the juice. The main sugars are fructose, sucrose, and glucose conveniently measured by refractive index as percentage of total sugars in the fruit juice (Brown and Harvey 1971).

Acidity and sweetness are inherited independently. From an analysis of over 100 cultivars, the majority of successful dessert apples are in the groups of medium acid/medium sugar, medium acid/high sugar, and low acid/medium sugar. The inheritance of sweetness, measured as the sugar concentration in the fruit juice, shows a quantitative pattern with a normal distribution of progenies about the mean that is very close to the mean value of the two parents. The inheritance of acidity is, however, more complicated in that two patterns are involved. A single gene control, with medium, to high acidity being dominant over very low acidity, is superimposed on a quantitative pattern. The very low acid seedlings, often referred to as "sweets," have from 0.1 to 0.3% malic acid in the fruit. In progenies where one or both parents are homozygous acid (*Ma Ma*) all the seedlings will have normal acidity. In other progenies, *Ma ma* × *Ma ma* or *Ma ma* × *ma ma,* a quarter or a half of the seedlings will be of the sweet type. These very low acid types are on the whole undesirable and have to be discarded.

In progenies where one or both parents are homozygous dominant, the distribution of acidity is normal with the progeny mean approximating the parental mean. In progenies where sweets are segregating and the sweets are excluded, the mean of the remaining acid portion is somewhat higher than the parental mean. When this is known, a fair estimate of the progeny mean for malic acid concentration can be made from the parental mean.

An unusual feature of this "sweet" character is that because of its unpleasant flavor it would have a negative selection value, yet few cultivars are known that are

homozygous for the dominant acid type and the majority of the cultivars studied are heterozygous. This suggests that this is an example where selection for quantitative economic characters has favored selection of heterozygotes for a neutral character (Williams and Brown 1956).

By knowing the sugar concentration and the malic acid concentration in the fruits of cultivars, parents can be selected that will produce progenies in which the majority of the seedlings will combine desirable sugar and acid contents. If the *Ma* genotype is known, the number of "sweets" that will have to be discarded can be predicted. If there is a selective advantage in the heterozygotes, then *Ma Ma* × *ma ma* progenies would all be heterozygous and such crosses would be worth considering. A comparison between a number of cultivars, their color sports, and their tetraploid sports showed no major differences in the sugar or acid concentration in their fruits.

Fruit Defects

A number of fruit and flesh defects have an important effect on apple quality. These include fruit cracking (Opara et al. 1996), calcium defects such as bitter pit (Ferguson and Watkins 1989), and the flesh disorder watercore, which results in a glassy appearance of the flesh (Marlow and Loescher 1984). Each of these defects is strongly influenced by genotype and environment. It is important that susceptibility to these fruit defects be considered in the selection process.

Fruit cracking varies by type (from cuticle cracks to cracks that deeply penetrate the flesh), intensity, and genotype. Cracking is also greatly affected by the water status of the plant. Severe cracking of susceptible cultivars can lead to unacceptable losses. The inheritance of cracking is undetermined.

Calcium defects can be controlled by Ca sprays or sometimes by fertilization but susceptible cultivars can result in serious production problems. Bitter pit-resistant seedlings had higher levels of Ca and B and lower levels of Mg and K in both fruit peel and leaf tissues than susceptibles. Two genes, *Bp-1* and *Bp-2,* controlling Ca accumulation and distribution within the fruit were hypothesized (Korban and Swiader 1984). Bitter pit is also strongly affected by rootstock, probably related to Ca uptake or translocation. 'M.7' and 'MM.106' rootstocks reduced bitter pit in 'Golden Delicious' in South Africa.

Watercore appears to be due to a change in membrane integrity associated with maturation and ripening, resulting in the accumulation of fluid in the intercellular spaces and elevated sorbitol concentrations. It is associated with elevated sweetness in the fruit and is therefore preferred by the Japanese market. In some cultivars the effect disappears in storage; in badly affected cases watercore leads to storage breakdown resulting in huge economic losses. Most cultivars are susceptible to varying degrees. Watercore can be severe in some new cultivars such as 'Fuji' and 'Williams' Pride'. Only a few cultivars may be considered completely resistant, e.g., 'McIntosh' is resistant and the disorder is uncommon in 'Golden Delicious'.

Disease Resistance

Over the centuries apples have been selected for high productivity, good flavor, attractive appearance, and long storage life, but in the process the innate resistance of species to many diseases (see Jones and Aldwinckle 1990) and pests has been lost. Commercial orchards require a tremendous amount of chemical control to produce high productivity and blemish-free fruit. However, resistances to most diseases and pests can be found in various cultivars, suggesting that apples could be improved genetically to contain multiple resistance for many diseases and pests. Originally this concept was suggested as a technique to reduce grower costs; however, consumer fear of chemical pesticides has created a market for fruits grown without pesticides by what has become known as the "organic" market. Organic growers attempt to eliminate certain chemical sprays and utilize other techniques, one of which is the use of genetic resistance. The problem is that it is highly unlikely to produce an apple that is resistant to all of the natural shocks to which apple is heir. At present, only very early disease-resistant apples, such as 'Pristine' can produce blemish-free f1ruits without fungicides. Disease resistance will be important not only for organic growers but also for most home owners and small orchards. In the future it may be possible to produce fruit without insecticides and miticides by a combination of resistance, some of which may be introduced by genetic transformation, and by other means such as traps and the introduction of beneficial insects. A new approach called integrated pest management (IPM) strives to reduce but not to eliminate the use of chemical sprays by various techniques, including monitoring, the use of beneficial insects, more careful attention to pesticide application to reduce the amount used, and the use of genetic resistance.

Many of those pathogens and pests for which host resistances have been discovered have shown capability for overcoming those resistances. For example, *Vf* resistance to *Venturia inaequalis* (apple scab) has been overcome in Germany (Parisi et al. 1993); major gene resistance to *Podosphaera leucotrichum* (powdery mildew) is rapidly overcome, sometimes within a single growing season; some strains of *Erwinia amylovora,* the fire blight incitant, are capable of causing disease in some *Malus* clones previously thought to be resistant. Although *Bt* genes for bisect resistance look promising in preliminary tests, experience with the "Dipel" type insecticide, crystal spore suspensions of *Bt,* strongly suggests that insects will be able to overcome such resistance in time (Cummins and Aldwinckle 1992). There are other problems. A given disease may have more than one causal agent and developing cultivars with resistance to one agent may not guarantee resistance to others. For example, *Phytophthora cactorum* has long been considered the agent of crown rot and collar rot of apples, but it now is clear that them are several *Phytophthora* species that attack apple rootstocks and produce similar symptoms and that there are specific host/pathogen interactions (Mircetich and Browne 1989; Jeffers and Wilcox 1990).

The apple breeder should aim to minimize the likelihood of such breakdowns of resistance. There seem to be two general options. One is to pyramid resistant genes

and the other is to aim for tolerance rather than immunity, because this type of resistance may be more durable (Kellerhals and Furrer 1994). Disease resistance breeding is difficult but not hopeless. Many resistances have held up for a very long time. For example, 'Delicious' continues to display useful resistance to fire blight; *Malus floribunda* 821 has shown scab resistance in the United States for over a 100 years, and woolly apple aphids continue to be held in check in many areas (but not all) with the moderate resistance obtained from 'Northern Spy'.

Apple scab. This disease, caused by the fungus *Venturia inaequalis,* is the most serious disease of the apple worldwide. It attacks leaves (causing defoliation in severe cases) and shoots and disfigures the fruit to the point of unsalability. It can be controlled chemically but at considerable expense and difficulty. Breeding resistant cultivars is a rational strategy that has been attempted on a large scale for many years in many countries; new cultivars that are virtually field immune are now available.

Two forms of resistance are available: polygenic and monogenic. The most desirable and durable type of resistance is likely to be obtained from a combination of both types. As with most diseases, some cultivars are much more resistant than others, which allows selection for high, but not complete, resistance. A great many biotypes of the pathogen exist and when cultures are grown from single spores and tested on young leaves of cultivars some give positive and some negative results. However, resistance that does not act against races is unreliable. An excellent review of scab, the organism, and resistance strategy is found in MacHardy (1996).

Polygenic resistance is found in the cultivar 'Antonovka Poltobutanaja', which at one time was field resistant to all known races of the fungus but is now attacked by some races. This type of polygenic resistance is also found in selections of some species, notably *M. baccata, M. sargentii, M. sieboldii,* and *M. ×zumi calocarpa* (Shay et al. 1962). Forms of 'Antonovka' and selections from its progenies have been used extensively in breeding programs, particularly in Europe.

Modern breeding programs for scab resistance date to the discovery by L.F. Hough of field immunity to scab in progenies derived from species crosses made early in the century by C.S. Crandall at the University of Illinois (Crosby et al. 1992). A cooperative program was initiated by L.F. Hough at the University of Illinois and later Rutgers University and J.R. Shay at Purdue University and joined subsequently by E.B. Williams, J. Janick, J.A. Crosby (Purdue); D.F. Dayton and S.S. Korban (Illinois); and J. Goffreda (Rutgers) among others. This program spawned a number of independent disease-resistant programs, including those at Cornell University in the United States and in Australia, Brazil, Canada, Czechoslovakia, England, France, Germany, Hungary, Italy, and Romania. Species such as *M. micromalus, M. atrosanguinea, M. prunifolia,* and others have been introduced into the program, but the most advanced material carries the *floribunda* resistance. Some of this material has been shown to contain resistance to a number of other diseases, including fire blight, cedar-apple rust, or mildew with some tolerance to insects and mites. Some selections, such as 'Enterprise' and 'Liberty', show multiple disease resistance. At the present time about 70 cultivars from various

programs around the world have been introduced based on the *Vf* gene and a number show promise as potentially important cultivars, including 'Enterprise', 'GoldRush', 'Jonafree', 'Liberty', and 'Pristine'. At present the most widely planted scab-resistant cultivar is 'Judeline', a juice apple with more than a million trees in France.

The conventional method of testing for scab resistance is to grow seedlings in a greenhouse at a temperature of 18 to 20°C. When the first true leaves are showing between the cotyledons, the seedlings are sprayed with a spore suspension of mixed inoculum of the fungus and covered with polyethylene sheeting for 48 h or misted in a chamber to ensure that the leaf surface does not dry. They are inoculated again after 10 days as a safeguard. Sporulation is soon evident on the susceptible seedlings, which can be discarded. Satisfactory infection can be produced only on very young leaves. The inoculum is prepared by washing the conidia from laboratory cultures of all races of *V. inaequalis* plus isolates collected from many sources to give a spore suspension of a representative mixture of the pathogen. Races and isolates may also be built up separately on young susceptible seedlings and the conidia washed off the leaves. Conidial suspensions may be stored frozen for up to one year. Resistance to leaf infection is generally well correlated with resistance to fruit infection.

The resistance is scored according to symptoms on young leaves grown under greenhouse conditions. 0 = no macroscopic evidence of infection; 1 = pinpoint pits, no sporulation; 2 = irregular chlorotic or necrotic lesions and no sporulation; 3 = few restricted sporulating lesions; and 4 = extensive, abundantly sporulating lesions. The class M has been added to indicate a mixture of necrotic, nonsporulating, and sparsely sporulating lesions (Shay and Hough 1952a). Only class 4 is considered as field susceptible—all the others are classified as field resistant and do not show symptoms of infection when grown outdoors where conditions for scab are very favorable. Using a broad definition of resistance, crosses of heterozygous resistant with homozygous susceptibles segregate 1 resistant : 1 susceptible but usable resistance (completely nonsporulating, i.e., clones 0, 1, and 2) is lower, typically 30%. Through a series of intercrosses (tests of allelism) it was established that 11 of 25 sources of resistance were due to the same gene (*Vf*), found in *M. floribunda* 821. A total of six loci for qualitative resistance (defined gene pools) were identified as follows:

Symbol	Original source
Vf	*M. floribunda*
Vm	*M. micromalus*
Vr	*M. pumila* R12740-7A (a Russian apple; perhaps *M. sieversii*)
Vbj	*M. baccata jackii*
Vb	Hansen's baccata #2
Va	'Antonovka' PI 172623

Five different virulent races of *Venturia inaequalis* were identified up to 1993 and race 6 was recently discovered in Germany (Parisi et al. 1993):

Race	Source	Susceptible material
1	Worldwide	Most of the world's cultivars
2	South Dakota, USA	*M. baccata*, 'Dolgo', 'Alexis', 'Bittercrab' segregates of R12740-7A, 'Geneva'
3	Nova Scotia, Canada	'Geneva'
4	Lafayette, Indiana, USA	Segregates of R12740-7A
5	Norwich, England	*Micromalus* pit type resistance, *M. atrosangunia* 804
6	Ahrensburg, Germany	'Prima' (*Vf* cultivars) but not 'Evereste' *M.* דPerpetu" and *M. floribunda* 804

The isolates of race 6 examined to date are nonaggressive and have not increased in Ahrensburg, but the future of *Vf* is clouded. It is not clear if race 6 is a recent mutation or merely a biotype that has always existed. The appearance of this race underscores the necessity of combining qualitative and quantitative genes and to carefully eliminate any seedlings with type 3 reactions. The class 2 reaction has always remained field immune until the appearance of race 6. Previous reports of low levels of scab infection on *Vf* material had proven to be a result of mislabeling. But 'Prima', the first scab-resistant cultivar, exhibited only a 3 reaction type, and limited leaf sporulation of *V. inaequalis* can occur in some genotypes in some environments. At present, literally millions of *Vf* trees in France (principally the cultivar 'Judeline', a juice apple in France) remain scab-free.

Mildew. This scourge of apple is incited by *Podosphaera leucotricha.* Mildew is one of the most important diseases of apple, especially in humid climates. Although it usually does not damage the fruit to any extent, it debilitates the tree and can kill seedlings. It is a significant problem in nurseries and stoolbeds. This disease, which attacks the foliage and young shoots, has a very marked effect on the quantity and the quality of the fruit produced. Mildew varies in its intensity according to the climate, but is present wherever apples are grown. Some cultivars are very susceptible, others are highly resistant, and between these two extremes there is a complete range. 'Delicious', 'McIntosh' and the 'Geneva 65' rootstock, for example, are field resistant, while 'Idared', 'Jonathan' and the Malling-Merton rootstocks are extremely susceptible. It is doubtful if stable complete immunity can be found within the cultivated apple. It is possible, however, to select some that are very highly resistant and only rarely produce any infected shoots.

Mildew resistance can be inherited quantitatively with no evidence of dominance (Brown 1959). There is evidence that direct assessment of mildew resistance among cultivars provides a sufficiently reliable guide to their breeding behavior, but the differences in resistance are not clear until the seedlings are 2 years old.

McIntosh and Lapins (1966) found large differences in susceptibility to mildew among 21 clones of 'McIntosh' grown from dormant scions X-rayed at doses from 3.75 to 5 krad. Most showed normal susceptibility, but a few were very susceptible and some were quite resistant. The changes are attributed to mutations.

In addition to polygenic resistance, single gene immunity has been reported from *M. ×robusta* and *M. ×zumi* (Knight and Alston 1968, 1972; Alston 1977), and from an unknown interspecific: hybrid from Morton Arboretum 8, *M. ×zumi calocarpa, M. sargentii,* and *M. baccata jackii* (Dayton 1977; Korban and Dayton (1983). However, these monogenic resistances have succumbed to different pathogenic races of the fungus. Recently, there has been renewed interest in mildew-resistance breeding using both conventional (Janse et al. 1994; Kruger 1994; and Schmidt 1994) and new methods (Battle and Alston 1994).

Apple canker. Branch- or stem-encircling cankers caused by *Nectria galligena* Bres are on the increase and create considerable concern among fruit growers. On heavy soil in particular, trees seem prone to attack. To date, few investigations have been carried out on this disease, but at least it is known that there are considerable differences between cultivars in their susceptibility. Some cultivars, particularly among the cider apples, are claimed to be very highly resistant (Wormald 1955) as are 'M.1' and 'M.12' among the rootstocks (Moore 1960). Where degrees of resistance exist, breeding and selection could yield a high degree of resistance combined with good commercial qualities. Canker resistance has been a major objective in the Ahrensburg. Germany, breeding program (H. Schmidt, pers. comm.).

Valsa canker, caused by *Valsa ceratosperma* (Tode ex Fr.) Maire, is one of the serious apple diseases in Japan and none of the cultivars presently grown are resistant. Moderate resistance to valsa canker has been located in *M. sieboldii* (Bessho et al. 1994).

Fire blight. A serious bacterial disease caused by *Erwinia amylovora,* fire blight can infect most of the Pomoideae. It can be a devastating disease of pears and, although it is usually less severe on apples, no cultivar is immune. Once confined to the United States, fire blight is spreading to most of the apple world. The bacterium attacks flowers, fruit, shoots, and branches, and the susceptibility of any tree depends on the condition of the growth, tune of flowering, and environmental conditions at the time infective material is present. This can lead to conflicting reports on the susceptibility or resistance of a cultivar, although this may also be due to the existence of different biological races (Nonnecke 1948).

All commercial cultivars of apple are susceptible if inoculum. from a virulent culture of the bacterium is injected into succulent tissue, but immunity is known in some Maims species. There can be considerable resistance to the rapid advance of the disease into the tissue in some cultivars and very high resistance has been found in Robusta 5 (*M. ×robusta*) and *M. fusca* H-12. 'Splendour' is nearly immune to the disease. 'Delicious' and 'Winesap' are among the most resistant cultivars, while 'Jonathan', 'Rome Beauty', and 'York Imperial' are highly susceptible, as well as

some of the newer cultivars such as 'Fuji' and 'Gala'. Interestingly, a number of scab-resistant selections such as 'Prima', 'Priscilla', 'Enterprise', and 'Liberty' have a useful level of resistance; it is likely that resistance was transmitted fortuitously from *M. floribunda* 821 along with scab resistance. Yet, a number of scab-resistant selections are extremely susceptible; an otherwise promising selection, Co-op 25, was not released for this reason.

There are a number of ways of assessing the susceptibility of seedlings but most involve inoculation of the tip of actively growing terminal shoots and, after a given time, measuring the distance the disease has invaded the shoots and comparing the run of infection. It seems that a satisfactory resistance to fire blight, although not immunity, can be obtained through careful choice of parents and active screening in areas with erratic blight. In the U.S. Midwest, susceptible seedlings seldom escape blight in the field and most susceptible selections become identified naturally.

Fire blight is extremely important in rootstocks because basipetal transmission of the organism from flower and shoot infection through the trunk can kill the tree. Several clonal rootstocks including 'Alnarp 2', 'M.9', 'M.26', and 'Ottawa 3' are extremely susceptible to *E. amylova*. This poses a potential calamity in southern Europe where 'M.9' is almost universal. In the Geneva program, screening for fire blight resistance is a top priority. Fire blight may limit the deployment of very susceptible cultivars, such as 'Gala', on 'M.26' in some regions, of the United States including the Shenandoah Valley, Utah, and the Columbia Basin.

The possibility of obtaining resistance by genetic transformation is under investigation at Cornell University, Geneva. Results indicate that substantial resistance can by induced by transfer of the *attacin E* gene from the giant silk moth, *Hyalophoras cecropia* (Norelli et al. 1994).

Crown rot. This disease is caused mainly by the fungus *Phytophthora cactorum* (Leb. Cohn) Schroet. in many temperate regions but other species of *Phytophthora* are involved in certain areas. Unfortunately, resistance to *P. cactorum* does not necessarily indicate resistance to other species. Zoospores in the soil infect the bark of apple trees at or new ground level and it is possible to avoid infection of the scion by grafting about 60 cm above soil level. The disease is capable of killing quite large trees by girdling the main stem especially in poorly drained areas. Since both rootstock and the scion cultivar may be attacked, it is essential to have resistant rootstocks and desirable to have resistant cultivars. *Phytophthora* resistance would be essential in those selections where self-rooted trees could be of interest.

There appears to be both polygenic and monogenic resistance. McIntosh and Mellor (1954), using a mixed inoculum to infect trees, suggested that resistance is partially or completely donibmt. Resistant × resistant progenies produced 7% infected seedlings; resistant × susceptible 24%, and susceptible × susceptible 93%. Alston (1970a) reported a single dominant resistance gene (*Pc*) in 'Northern Spy'. The moderate resistance to collar rot in some of the Mailing-Merton rootstocks is derived from 'Northern Spy'. Sources of resistance among dwarfing rootstocks

include 'M.9', 'M.27', and 'Ottawa 3', but a higher level of resistance is transmitted by *M. ×sublobata* 'Novole', *M. angustafolia,* and *M. ×magdeburgensis* Hartwig (Cummins and Aldwinckle 1983). However, there are great differences in pathogenicity between fungal isolates.

A very rapid method for the preselection for collar rot resistance is described by Watkins and Werts (1971) and later modified by Cummins and Aldwinckle (1983), where young seedlings 2 to 3 weeks old are grown in a peat-sand mixture that is inoculated by flooding with a mixture of zoospore suspensions. The effects of attack are wen after 1 week and the complete results after 2 weeks. By this method, only the resistant or tolerant ones are left to grow on for further testing. In the Cornell University, Geneva, rootstock program, 15 to 30% of the seedlings inoculated with *P. cactonan* and *P. megasperma* survive inoculations, flooding, and incubation. Other methods of testing cultivars and seedlings usually depend on culturing the fungus in the laboratory and then inoculating with mycelium, infected agar disks into the stem of growing trees or into cutoff shoots in controlled conditions in the greenhouse or laboratory and after a given time measuring the infection (Sewell and Wilson 1959). These methods probably reflect only certain portions of a plant's response to *Phylophthora* in an orchard setting.

Apple rust*.* The cedar apple rust fungus, *Gymnosporangium juniperi-virginianae* Schw., attacks leaves and fruits of apple and has the red cedar (*Juniperus virginiana* L.) as its alternate host. It has been quite serious in the eastern United States. Quince rust, hawthorne rust, and a related rust species in Japan can be serious problems. A number of high-quality cultivars are resistant. Moore (1940) and Shay and Hough (1952b) considered resistance to be controlled by a single dominant gene, with 'Arkansas Black' and 'McIntosh' being homozygous for resistance; 'Delicious', 'Winesap', and 'Wolf River' heterozygous; and 'Jonathan' and 'Rome Beauty' fully susceptible. However, Mowry (1964), Aldwinckle et al. (1977), and Chen and Korban (1987) consider resistance to be controlled by two dominants with the double recessive susceptible. 'Priscilla' was considered to be homozygous dominant for resistance (Aldwinckle et al. 1977); 'Golden Delicious' is susceptible.

Apple blotch*.* Mowry and Dayton (1964) analyzed the progenies from controlled crosses for susceptibility to blotch caused by *Phyllosticta solitaria* E. & E. Susceptibility was controlled by two completely dominant genes with duplicate recessive epistatic interaction between the gene pairs. It was not possible to predict with any accuracy the genotype of a cultivar from its expressed apple blotch phenotype.

Glomerella leaf blotch*.* 'Golden Delicious' was reported heterozgyous for a dominant gene *Gb* which confers susceptibility to a new strain of *Glomerella*

cingulata (Stonem.) Spauld. & Schrenk. Camilo (1989) found all isolates pathogenic to every Malus clone except *M. ×sieboldii* 2892-22.

Storage rots. Considerable losses can occur from fruit rotting diseases in storage, and species of *Gloeosporium,* particularly *G. perennans* Zellar & Childs, are largely responsible. In a survey of over 200 cultivars, Alston (1967) found that some are very susceptible and a few (about 5%) were highly resistant, with 'Cravert Rouge' the most resistant. A small progeny from 'Cox's Orange Pippin', which is very susceptible, crossed with 'Cravert Rouge' produced seedlings with a fair degree of resistance. When compared with a progeny of 'Cox's Orange Pippin' selfed which had a mean of 4.7 lesions per fruit, the progeny from the cross with 'Cravert Rouge' had a mean of 0.8 lesions. Also among the most resistant cultivars in this survey were 'Jonathan' and its color sport 'Jonared', and a number of derivatives of 'Jonathan' also show good resistance to this pathogen. The method of estimating the susceptibility is to dip the fruits in a spore suspension (50,000 spores/mL) and store in paper bags at 5°C for up to 5 months. Five or more fruits of each cultivar need to be sampled.

Virus diseases. Diseases caused by viruses, viroids, and mycoplasm-like organisms present a number of problems to the fruit grower and the propagator since many (like tomato ringspot virus, rubbery wood, and flat limb) are quite serious and my disable the tree; others affect the efficiency of the tree, and most cause a reduction in crop. Viruses pass freely across graft unions, so it is important to have virus-free rootstocks as well as virus-free fruiting cultivars. The responses of apple cultivars to viruses differ widely. Some viruses are present in many cultivars without showing any symptoms. With others, such as vein-banding mosaic, the severity of the symptoms varies from one cultivar to another, some being very severe, some moderate, some slight, and some symptomless (Luckwill 1953). Other viruses produce severe symptoms or none at all. Many of the viruses have been present but undetected in cultivars until fairly recent times when indicator plants have been found that produce visible symptoms (Fridlund 1989). Most virus can now be detected by enzyme-linked immnunosorbant assays (ELISA).

Since so many cultivars carry viruses without showing symptoms, it would be an almost impossible task to try to breed for resistance. There is evidence that sensitivity my be passed to offspring. In the scab breeding program 'Russian R-12740-7A', which is an indicator for chlorotic leaf spot, passed on the sensitive reaction to some of its seedlings (Mink and Shay 1962). The vole-resistant apple rootstock 'Novole', which is sensitive to apple stem-grooving virus, transmit this sensitivity to more than half its seedlings. The incorporation of virus resistance into plants is one of the successes of genetic engineering (Grumet 1994) and this approach is under investigation in apple (Mauren et al. 1992). The rootstock 'MM.106' is very susceptible to tomato ringspot virus; genetically engineered resistance is being developed at Geneva (D. Gonsalves, personal communication).

Pest Resistance

Resistance breeding to insect pests began in response to epizootics of woolly apple aphid in Australia, New Zeal-I South Africa and the southeastern United States. Woolly apple aphis *(Eriosoma lanigerum* Hausm.) is a very widespread pest of apples that is capable of attacking not only the above-ground stem of the tree, causing cankers, but also die roots, where large colonies can become established under certain conditions. The above-ground infestations can be controlled by pesticides, but those on the roots cannot. Woolly apple aphis can be quite a serious problem on nursery stocks especially in the stoolbed. Breeders at the John Innes Institute and East Malling Research Station in England and at the United States Department of Agriculture began breeding for resistance in the early 1920s. Growers had recognized for some time that 'Northern Spy' and some of its derivatives, including 'Ivorys Double Vigour', were resistant to the woolly apple aphid and were more or less satisfactory for use as rootstocks. Subsequently, Cummins et al. (1981) also identified 'Kola', *M. halliana*, *M. hupehensis*, *M. ×robusta* ('Robusta 5'), and *M. tschonoskii* as resistant. Resistance found in 'Irish Peach', 'Malling 15' and *M. ×micromalus* is not transmitted to progeny, at least not in the F_1.

In England, 'Northern Spy' was crossed with 'M.1', 'M.2', 'M.9', and 'M.15' to produce progenies that were rigorously screened for resistance. The USDA breeders similarly resorted primarily to 'Northern Spy' for resistance. The American program produced 'Spy 227'. much used today by virologists to detect apple stem-pitting virus. The Merton Immune series was produced from the English program, of which 'Merton Immune 793' remains in use today, while 'MM.106' and MM.111' the Malling-Merton rootstocks, have gained world-wide acceptance.

Resistance has also been found to the rosy apple aphid (*Dysaphis plantaginea* Pass.) and the rosy leaf-curling aphid, which are conditioned by different single dominants (Table 4). Resistance to the rosy leaf curling aphid (*Dysaphis devecta* Wlk.) is controlled by a single dominant gene, Sm_h, obtained in an open pollinated selection of *M. ×robusta*. Alston and Briggs (1970) found three biotypes of the rosy leaf-curling aphid and at least three resistant genes in the host. 'Cox's Orange Pippin' carries Sd_1 and is resistant to biotypes 1 and 2; 'Northern Spy' has Sd_2 and is resistant to biotype 1 only; and a selection from *M. ×robusta* with Sd_3 is resistant to all three biotypes.

Goonewardene et al. (1998; Goonewardene and Williams 1988) screened progenies, accessions, and scab-resistant selections of Malus and found several selections and progenies with multiple pen and disease tolerance or resistance from observations in the field, laboratory, and greenhouse. The arthopods investigated included apple maggot, *Rhagoletis pomonella* (Walsh); codling moth, *Laspyresia pomonella* (L.); European red mite, *Panonychus ulmi* (Koch.); plum curculio, *Conotrachelus nenuplar* (Herbst); and redbanded leafroller, *Argyrotaenia velutinana* (Walker). Insect resistance was typically compared by the results of differential feeding on "June-drop" apples as compared to a control. Seven apple selections (E11-24, E14-32, E36-7, D7-47, E7-54, E29-56, and E31-10) have been released as advanced germplasm lines with multiple resistance to pests and diseases

(Goonewardene 1987; Goonewardene and Howard 1989) and are presently under evaluation.

Results from the transformation of apple with *Bt* genes (see Transformation) indicate that resistance to lepidopterous bisects, including codling moth and perhaps apple maggot, may be obtained. It is uncertain whether the ultimate strategy will be to add them to new cultivars or to develop trap trees (e.g., by incorporating into crab pollinators) that will be used to reduce the population levels of the insect in commercial orchards (Dandekar, personal communication). Clearly, recent advances in biotechnology are opening up a new em in insect resistance of apple.

Rootstock Breeding

The rootstock is a critical part of the apple tree, and rootstock breeding must be considered with as much care as that of the scion cultivars. Rootstocks must satisfy many special requirements and the testing period is long and difficult, for it is only when the scion is grafted on the rootstock and has reached full cropping that the potential of the rootstock can be assessed. World breeding programs have been reviewed by Cummins and Aldwinckle (1983) and Ferree and Carlson (1987).

There are a number of special requirements for any rootstock: ease of propagation either vegetatively or by seeds; clean upright stern, easy to bud or graft; a root system that will provide adequate anchorage to support the tree without staking; and absence of stock-scion incompatibility, suckers, and burrknots (root primordia on the trunk). Rootstocks should induce early and heavy cropping and be resistant to a number of pests and diseases. Finally, specific rootstocks should offer a range of tree size control. The objectives of the Cornell University, Geneva, apple rootstock breeding program are shown in Table 6, but specific objectives can be expected to vary in other parts of the world. For example, rootstocks my have to be extremely winter hardy, adapted to alkaline or waterlogged soil to increase calcium uptake to avoid fruit defects such as bitter pit, or offer resistance to specific root diseases, e.g., black root rot (caused by *Xylaria mali* Fromme), limited to the southern United States; Rosellinia root rot (*Rosellinia necatrix* (Hart.) Berl.), important in India and California, and *Sclerotum rolfsii* Sacc., important in Israel and other subtemperate regions. At present there is a wide range of vegetatively propagated apple rootstocks that impart different degrees of vigor, ranging from those producing dwarf, compact trees to those producing very large, vigorous trees, but there is a need for a greater range in winter-hardy stocks and for resistance to many pests and diseases.

One of the problems of vegetatively propagated rootstocks is virus diseases, which may be present in the rootstocks and then passed to the scion. One way of overcoming this is to raise rootstocks from seeds. This practice has been in use for a great many years and one of the reasons for the change to clonal stocks was the great lack of uniformity among the seedlings, since those used as rootstocks displayed the enormous variability found in most apple seedling populations. In recent years apomictic seedlings, which are much more uniform, have been bred from some *Malus* species. These, too, present problems; some exhibit extreme sensitivity to

TABLE 6. Objectives of Cornell University, Geneva Apple Rootstock Breeding Project

Attribute	Minimum standard	Ideal standard
Essential		
Resistant to *Erwinia amylovora* (fire blight)	M.7	R5
Resistant to *Phytophthora* spp. (crown rot)	M.9	Bud.9
Readily propagable	MM.106	A.2
Liners smooth, relatively free of spines	MM.106	O.3
Liners thrifty in nursery row; easily buddable	MM.106	A.2
Induce heavy, early fruit production	MM.106	M.9
Early hardening of scion and collar	M.2	M.9
Tolerant to low midwinter temperatures	MM.111	A.2
Late-leafing in early spring	M.9	K-14
Free of burrknots	M.2	Hibernal
Reacting to TmRSV as does	M.7	Delicious
Induce no more scion susceptibility to *E. amylovora* than	M.7	
Important		
Resistant to woolly apple aphids	MM.111	R5
Roots structurally strong (not brittle)	M.26	M.7
Well anchored	M.7	M.111
Few or no suckers in orchard	MM.106	Hibernal
Inducing early maturation of fruit	M.2	M.9
Resistant to pine voles	R5	Novole
Hardy to very low temperatures in midwinter	M.26	R5
Tolerant of chlorotic leaf spot, apple stem grooving, and apple stem pitting viruses	M.9	M.9
Helpful		
Leaves red or otherwise distinctive	Bud.9;O.3	
Moderately resistant to *Venturi inaequalis*	M.7	
Moderately resistant to *Podosphaera leucotricha*	M.9	
Resistant to meadow vole	Sugar Crab	
Special Objectives		
Tree size control: full range, from dwarfing through vigorous	M.9 Alnarp 2	
Some stocks tolerant of poorly drained soils	M.13	
Some stocks tolerant of drought	MM.111	

virus infection from the scion. They have the advantages that propagation is simple and the seedlings are uniform and free from virus. A number of species have been tried, particularly *M. sieboldii, M. sargentii, M. hupehensis, M. sikkimensis, M. toringoides,* and hybrids between some of these. *M. hupehensis* and *M sikkimensis* are autonomous apomicts and can produce apomictic seeds without pollination,

which produce seedlings of the maternal type. Other species are facultative apomicts and a small percentage of the seedlings may be hybrids. Since sour of these require pollination to produce seeds, they can be pollinated with a homozygous red-leafed plant and the undesirable hybrids immediately identified by the pigmentation of the leaves. When virus-free clones are used as scions much of the incompatibility disappears. Some *M. sieboldii* hybrids have shown a good affinity for virus-infected scions and appear tolerant of latent virus infection (Schmidt 1970).

Evaluating vegetatively propagated rootstocks is by its nature a difficult and long-term project. In the initial stages, each new seedling must be tested to determine its ability to root and to produce a good stem, its rate of multiplication, and whether it has all the normal qualities that make a good healthy plant. If disease or pest resistance is being incorporated, this can be determined at this stage. But final testing of new candidates must be carried out in many areas to determine adaptability. In North America, regional programs (NC 140) have been organized to evaluate rootstock-scion (stionic) interactions with location.

ACHIEVEMENTS AND PROSPECTS

The apple is an ancient crop that has been under rather continuous selection since antiquity. Until very recently the apple industry has depended on nurseryman and fruit-grower selections of scion cultivars and rootstocks with about a dozen genotypes dominating the commercial markets of the world. Deficiencies inherent in these genotypes in many cases have been ameliorated through cultural practices to prop them up, including specific rootstocks combinations, insect and disease control via chemical pesticides, growth regulators, and special handling and storage technology.

Plant breeding did not contribute significant new cultivars to the industry during the first half of the twentieth century but in the last half, the results of a number of breeding efforts around the world have begun to show an impact and the traditional cultivars are slowly being replaced. There is now an increasing demand for new selections produced by conventional breeding and a number of extremely promising selections are under test throughout the world. Some of these contain resistance to various diseases; a number have greatly improved storage life, improved textural quality, winter hardiness, and/or different tree forms. Some of these genotypes together with intensive methods of production, particularly high-density orchards, can be expected to have a profound effect on the future apple industry of the world.

The selection of superior genotypes from genetically variable populations derived from sexual recombinations is powerful because it is evolutionary and progress can be cumulative, with improved individuals continually serving as parents for subsequent cycles of breeding. Thus, genetic improvement of apple has made substantial changes when the effort has been well supported and long term. Successful programs are now underway in the United States, Japan, Australia, New Zealand, and various countries of Europe.

However, there have been restraints to conventional plant breeding that are especially limiting in tree fruits with long juvenile period and that are represented by unique, highly selected genotypes. These include the reliance on naturally occurring variation which may be unavailable, or more likely found in very primitive and unadapted material; restraints due to the inability of the sexual system to incorporate genes from nonrelated species and especially the inability of the sexual system to incorporate small changes without recombination resulting in the loss of desirable unique combinations; the difficulty of selection in detecting infrequent or rare recombinations; and the dependence of conventional breeding upon time to generate cycles of recombination, space to grow the necessary population to recover superior recombinants, and resources to be able to select, identify, and evaluate desirable recombinants.

Some of these limitations may be overcome by novel strategies from advances in biotechnology. Recombinant DNA has been especially attractive to fruit breeders because it offers a way to overcome the limitations of the sexual system by permitting the introduction of foreign genes heretofore unusable and more important makes it possible to introduce small discrete defined changes into established genotypes. The use of molecular markers will facilitate selection and provide the ability to identify clones with a great deal of precision for cultivar protection and perhaps to tag clones with a genetic brand.

Recent advances by a new generation of fruit breeders have demonstrated that these new technologies are feasible in apple. The transfer of genes effective against fire blight and insertions of genes from *Bacillus thuringensis* that may control lepidopterous pests such as codling moth have been achieved and are under test. The future promises to be very exciting. However, it remains clear that these new technologies will not replace but will merely complement conventional breeding. Most of the advances in DNA transformation will in the end involve improvements due to the insertions of single genes, which represent a conservative approach to improvement. Truly transgressive changes must still rely on sexual recombination.

How will breeders meet the challenges that are required? At present most are employed by government or state research stations or by universities where resources to continue large programs are becoming restricted. The private sector, particularly nurseries selling apple stock, has not gone into apple breeding, preferring to scavenge naturally occurring variation and sports from growers and to distribute selections from public sector breeding. What is clearly needed are new levels of cooperation between breeding programs within and between countries and between the public and private sector. The cooperative disease-resistant breeding program between Purdue University, Rutgers University, and the University of Illinois, which has been active for over 40 years is a good example, but the future is clouded by the level of individual support from each university. In Europe, three new cooperative efforts may stand as good models for the future: (1) Apple breeders at INRA and private nurseries; (2) apple breeders at the Institute for Horticultural Research at East Malling are strongly underwritten by a group of large European and American

nurseries; (3) apple breeding in Belgium with cooperation between University breeders and private nurserymen.

The acceptance of plant patenting and the return of royalties (some of which nay be returned to the breeding program) is now firmly entrenched in the public sectors, since research funds for long-term program have become limited or disappeared. But plant variety protection and the lure of royalties while engendering cooperation between the public and private sector could have the unhappy consequence of restricting, discouraging, or impeding cooperation among breeders. A new wrinkle of cultivar licensing has occurred in parts of the fruit industry where new cultivars are restricted to very large growers and royalties are returned in the form of a surcharge on each box of fruit (Janick 1995). This could have a profound effect on plant breeding.

Despite all of these obstacles and problems it is clear that the future of apple breeding will continue to be exciting with the introduction of new technologies, new genes, and new cultivars. Clearly, apples will continue to be one of the most popular fruits of humans, and plant breeders will have a key role in this industry.

ACKNOWLEDGMENTS

LITERATURE CITED

Abbott, D. L. 1955. Temperature and the dormancy of apple seeds. p. 746-753. In: Rep. Int. Congr. Scheveningen.

Aldwinckle, H. S., H. L. Gustafson, and R. C. Lamb. 1976. Early determination of genotypes by forced flowering of test cross progenies. Euphytica 25:185-19 1.

Aldwinckle, H. S., R. C. Lamb, and H. L. Gustafson. 1977. Nature and inheritance of resistance to *Gymnosporangium juniperi-virginianae* in apple cultivars. Phytopathology 67:259-266.

Alston, F. H. 1967. Varietal response to *Gloeosporium perennans* in the apple. p. 132-134. In: Rep. E. Malling Res. Sta. 1966.

Alston, F. H. 1970a. Resistance to collar rot, *Phytophthora cactorum* (Leb. and Cohn) Schroet. in apple. p. 143-145. In: Rep. E. Malling Res. Sta. 1969.

Alston, F. H. 1970b. Integration of major characters in breeding commercial apples. p. 231248. In: Proc. Eucarpia Fruit Breeding Symp., Angers.

Alston, F. H. 1973. Apple scion varieties: p. 134. In: Preliminary selection. Annu. Rep. E. Malling Res. Sta. 1972.

Alston, F. H. 1976. Dwarfing and lethal genes in apple progenies. Euphytica 25:505-514.

Alston, F. H. 1977. Practical aspects of breeding for mildew (*Podosphaera leucotricha*) resistance in apples. p. 4-13. In: Proc. Eucarpia Symp. Tree Fruit Breeding, 1976, Wageningen.

Alston, F. H., and J. B. Briggs. 1968. Resistance to *Sappaphis devecta* (WIk.) in apple. Euphytica 17:468-472.

Alston, F. H., and J. B. Briggs. 1970. Inheritance of hypersensitivity to rosy apple aphid *Dysaphis plantaginea* in apple. Can. J. Genet. Cytol. 12-257-258.

Alston, F. H., and J. B. Briggs. 1977. Resistance genes in apple and biotypes of *Dysaphis devecta.* Ann. Appl. Biol. 97:75-81.

Alston, F. H., and L. D. Hunter. 1971. Scab resistance and the hydroxy phloridzin, sieboldin. p. 95. In: Annu. Rep. E. Malling Res. Sta. 1970.

Aronson, A. I. 1994. *Bacillus thuringensis* and its use as a biological insecticide. Plant Breeding Rev. 12:19-45.

Barrett, H. C., and T. Arisumi. 1952. Methods of pollen collection, emasculation and pollination in fruit breeding. Proc. Am. Soc. Hort. Sci. 59:259-262.

Battle, L., and F. H. Alston. 1994. Isoenzyme aided selection in the transfer of mildew (*Podosphaera leucotricha*) resistance from *Malus hupehensis* to the cultivated apple. p. 17-20. In: H. Schmidt and M. Kellerhals (eds.), Progress in temperate fruit breeding. Kluwer Academic, Dordrecht, Netherlands.

Battle, L., F. H. Alston, and K. M. Evans. 1995. The use of the isoenzymic marker gene Got-1 in the recognition of incompatibility S alleles in apple. Theor. Appl. Genet. 90:303-306.

Beakbane, A. B. 1941. Anatomical studies of stems and roots of hardy fruit trees, In: the anatomical structure of some clonal and seedling apple rootstocks stem- and root-grafted with a scion variety. J. Pomol. Hort. Sci. 18:344-367.

Beakbane, A. B., and P. K. Majumder. 1975. A relationship between stomatal density and growth potential in apple rootstocks. J. Hort. Sci. 50:285-289.

Beakbane, A. B., and E. C. Thompson. 1939. Anatomical studies of stems and roots of hardy fruit trees, 11: the internal structure of the roots of some vigorous and some dwarfing rootstocks and the correlations of structure with vigor. J. Pom. 17:141-149.

Beakbane, A. B., and E. C. Thompson. 1947. Anatomical studies of stems and roots of hardy fruit trees, 4: the root structure of some new clonal apple rootstocks budded with Cox's Orange Pippin. J. Pom. 23:206-211.

Bergendal, P. 0. 1970. On the inheritance of red color in crosses between 'Golden Delicious' and various red apple mutants. p. 181-194. In: Proc. Eucarpia Fruit Breeding Symp., Angers.

Bessho, H., J. Soejima, Y. Ito, and S. Komori. 1993. Breeding and genetic analysis of apple in Japan. In: T. Hayashi et al. (eds.). Techniques on gene diagnosis and breeding in fruit trees. FTRS, Japan.

Bessho, H., S. Tsuchiya, and J Soejima. 1994. Screening methods for resistance to *Valsa* canker. p. 49-52. In: H. Schmidt and M. Kellerhals (eds.). Progress in temperate fruit breeding. Kluwer Academic, Dordrecht, Netherlands.

Bishop, C. J. 1959. Radiation-induced fruit colour mutations in apples. Can. J. Genet. Cytol. 1:118-123.

Blazek, J. 1983. Inheritance and genetic variation of spurred growth habit in Apples. Acta Hort. 140:55-62.

Blazek, J., and F. Paprustein. 1988. Complex number for evaluation of apple breeding stock. Acts. Hort. 224:185-196.

Bolar, J. T. 1995. Examination of factors affecting the transformation of 'Marshall McIntosh' apple by *Agrobacterium tumefaciens.* Thesis, Cornell University, Ithaca, NY

Bondt, A. D., K. Eggermont, P. Druart, M. D. Vil, I. Goderis, J. Vanderlayden, and W. F. Broekaert. 1994. *Agrobacterium-* mediated transformation of apple *(Malus domestica* Borkh): an assessment of factors affecting gene transfer efficiency during early transformation steps. Plant Cell Rep. 13:587-593.

Bowman, H. G., and D. Hultmark. 1987. Cell-free immunity in insects. Annu. Rev. Microbiol. 41:103-126.

Bringhurst, R. S. 1983. Breeding strategy. p. 147-153. In: J. Janick and J. N. Moore (eds.). Methods in fruit breeding. Purdue University Press, West Lafayette, IN.

Brittain, W. H. 1933. Apple pollination studies in the Annapolis Valley, N. S., Canada. 1928-32. Can. Dept. Agric. Bull. 162.

Broothaerts, W., G. A. Janssens, P. Proost, and W. F. Broekaert. 1995. cDNA cloning and molecular analysis of two self-incompatibility alleles from apple. Plant Mol. Biol. 27:499-511.

Brown, A. G. 1954. Frost susceptibility of 2x and 4x Fertility pear. p. 7. In: 45th Annu. Rep. John Innes; Inst.

Brown, A. G. 1959. The inheritance of mildew resistance in progenies of cultivated apple. Euphytica 8:81-88.

Brown, A. G. 1960. The inheritance of shape, size and season of ripening in progenies of the cultivated apple. Euphytica 9:327-337.

Brown, A. G. 1964. Fruit breeding. p. 37-38. In: 55th Annu. Rep. John Innes Inst.

Brown, A. G. 1966. New fruits from old. p. 10-24. In: Fruit present and future. Royal Hort. Soc., London.

Brown, A. G. 1975. Apples, p. 3-37. In: J. Janick and J. N. Moore (eds.). Advances in fruit breeding. Purdue University Press, West Lafayette, IN.

Brown, A. G., and D.M. Harvey. 197 1. The nature and inheritance of sweetness and acidity in the cultivated apple. Euphytica 20:68-80.

Brown, S. K. 1992. Genetics of apple. Plant Breeding Rev. 9:333-365.

Camilo, A. P. 1989. Genetic resistance in *Malus* to *Glomerella cingulata* (Stoneman) Spaulding and Von Schrenck: Sources of resistance, leaf infection, progeny evaluation, and pathogenicity. Thesis, Cornell University, Ithaca, NY.

Chan, B. G., and J. C. Cain. 1967. The effect of seed formation on subsequent flowering in apple. Proc. Am. Soc. Hort. Sci. 91:63--68.

Chen, H., and S. S. Korban. 1987. Genetic variability and the inheritance of resistance to cedar-apple rust in apple. Plant Pathol. 36:168-174.

Chevreau, E., Y. Lespinasse, and M. Gallet. 1985. Inheritance of pollen enzymes and polyploid origin of apple (*Malus xdomestica* Borkh). Theor. Appl. Genet. 71:268-277.

Chyi, Y. S., and N. F. Weeden. 1994. Relative isozyme banding pattern permit the identification of the *2n* gamete parent for triploid apple cultivars. HortScience 19:818-819.

Compton, M. E., and R. E. Veilleux. 1992. Thin layer morphogenesis. Hort. Rev. 14:239-264.

Conner, P. J., S. K. Brown, and N. F. Weeden. 1995. Examination of apple (*Malus xdomestica*) fruit quality and tree and fruit morphology using molecular markers. HortScience 30:832 (Abstr. 448).

Crane, M. B. 1953. The genetics and breeding of tree fruits p. 687-695. In: Rep. 13th Int. Hort. Congr. London.

Crane, M. B. and W. J. C. Lawrence. 1933. Genetical studies in cultivated apples. J. Genet. 28:265-296.

Crosby, J. A., J. Janick, P. C. Pecknold, S. S. Korban, P. A. O'Connor, S. M. Ries, J. Goffreda, and A Voordeckers. 1992. Breeding apples for scab resistance: 1945-1990. Fruit Var. J. 46:145-166.

Cummins, J. N. and H. S. Aldwinckle. 1983. Breeding apple rootstocks. Plant Breeding Rev. 1:294-394.

Cummins, J. N. and H. S. Aldwinckle. 1988. New directions in rootstock breeding. HortScience 23: 100-101.

Cummins, J. N. and H. S. Aldwinckle. 1992. Resistant host plants-The too-often absent component of Integrated Fruit Production. Proc. Australian Soc. Hort. Sci. 1: 157-163.

Cummins, J. N., P. L. Forshne, and J. D. Mackenzie. 1981. Woolly apple aphid colonization on *Malus* cultivars. J. Am. Soc. Hort. Sci. 106:26-30.

Dandekar, A. M., G. H. McGranahan, and D. J. James. 1992. Transgenic woody plants. p. 129-151. In: Shain-dow and R. Wu (eds.). Transgenic plants, Vol. 2. Academic, New York.

Darlington, C. D., and A. A. Moffett. 1930. Primary and secondary chromosome balance in *Pyrus.* J. Genet. 22:129-15 1.

Dayton, D. F. 1959. Red color distribution in apple skin. Proc. Am. Soc. Hort. Sci. 74:7281.

Dayton, D. F. 1977. Genetic immunity to apple mildew incited by *Podosphaera leucotricha.* HortScience 12:225-226.

Dayton, D. F., and E. B. Williams. 1968. Independent genes in *Malus* for resistance to *Venturia inaequalis.* Proc. Am. Soc. Hart. Sci. 92:89-.94.

Decourtye, L. 1967. A study of some characters controlled by simple genetic means in apple trees *(Malus* sp.) and pear trees (*Pyrus communis*) (in French). Ann. Amelior. Plantes 17:234--266.

Dermen, H. 1949. Are the pomes amphidiploid? A note on the origin of the Pomoideae. J. Hered. 40:221-222.

Dermen, H. 1965. Colchiploidy and histological imbalance in triploid apple and pear. Am. 1. Bot. 52:353-359.

Downing, D. L. (ed.). 1989. Processed apple products. Van Nostrand Reinhold, New York.

Durham, R. E., and S. S. Korban. 1994. Evidence of gene introgression in apple using RAPD markers. Euphytica 79:109-114.

Eichholtz, D. A., H. A. Robitaille, and P. M. Hasegawa. 1979. Adventive embryony in apple. HortScience 14:699-700.

Einset, J. 1945. The spontaneous origin of polyploid apples. Proc. Am. Soc. Hort. Sci. 46:91-93.

Einset, J. 1952. Spontaneous polyploidy in cultivated apples. Proc. Am. Soc. Hort. Sci. 59:291-302.

Einset, J., and B. Imbofe. 1947. Chromosome numbers of apple varieties and sports. Proc. Am. Soc. Hort. Sci. 50:45-50.

Einset, J., and B. Imbofe. 1949. Chromosome numbers of apple varieties and sports, 2. Proc. Am. Soc. Hort. Sci. 53:197-201.

Einset, J., and B. Imhofe. 195 1. Chromosome numbers of apple varieties and sports, 3. Proc. Am. Soc. Hort. Sci. 58:103-108.

Ellis, R. H. 1985. Seed storage and germination of apple and pear. In: Long term storage of major temperate fruits. Int. Board Plant Genetic Resources, Rome.

Elobeidy, A., and S. S. Korban. 1988. The effect of thisdiazuron on shoot regeneration from apple leaf discs. HortScience 23:755.

Ely, S. 1993. The engineering of plants to express *Bacillus thuringiensis* d-endotoxins. p. 105-124. In: P. F. Entwistle (ed.). *Bacillus thuringiensis,* an environmental biopesticide. Wiley, New York.

FAO. 1995. 1994 production yearbook. United Nations, Rome.

Fasolo, F., R. H. Zimmerman, and I. Fordham. 1989. Adventitious shoot formation on excised leaves of invitro, grown shoots of apple cultivars. Plant Cell Tissue Organ Cult. 16:75-87.

Ferree, D. C., and R. F. Carlson. 1987. Apple rootstocks. p. 107-143. In: R. C. Rom and R. F. Carlson (eds.) Rootstocks for fruit crops. Wiley, New York.

Ferguson, I. B., and C. B. Watkins. 1989. Bitter pit in apple fruit. Plant Breeding Rev. 11:229-288.

Forsline, P. L. 1995. Adding diversity to the national apple germplasm collection: collecting wild apple in Kazakhstan. New York Fruit Quart. 3(3):3-6.

Forsline, P. L., E. E. Dickson, and A. D. Djangalieu. 1994. Collection of wild *Malus, Vitus* and other fruit species genetic resources in Kazakhstan and neighboring republics. HortScience 29:433.

Fridlund, P. R. (ed.). 1989. Virus and viruslike diseases of pome fruits and simulating noninfectious disorders. Coop. Ext. Publication SP003. Washington State University, Pullman, WA.

Gilbert, N. 1967. Additive combining abilities fitted to plant breeding data. Biometrics 23:45-49.

Gonzalez-Cepeda, I. A. 1992. The Mexican apple industry as it exists today. Compact Fruit Tree 25:95-98.

Goonewardene, H. F. 1987. El 1-24, E14-32 and E36-7 apple germplasm with multiple pest resistance. HortScience 22:1346-1348.

Goonewardene, H. F., and P.H. Howard. 1989. E7-47, E7-54, E29-56, and E31-10 apple germplasm with multiple pest resistance. HortScience 24:167-169.

Goonewardene, H. F., and E. B. Williams. 1988. Arthropod and disease resistance in selections of Malus sp. originating from the cooperative apple breeding program. Purdue Univ. Agric. Exp. Sta. Bull. 537, West Lafayette, IN.

Goonewardene, H. F., P. H. Howard, and J. Triscari. 1988. Arthropod and disease resistance in progenies of *Malus* sp. originating from the USDA (ARS)-Purdue program. Purdue Univ. Agric. Exp. Sta. Bull. 540, West Lafayette, IN.

Gray, J. E., S. Picton, J. J. Giovannoni, and D. Grierson. 1994. The use of transgenic and naturally occurring mutants to understand and manipulate tomato fruit ripening. Plant Cell Environ. 17:557-571.

Grumet, R. 1994. Development of virus resistant plants via genetic engineering. Hort. Rev. 12:47-79.

Hagens, D. M. 1992. Genetic studies in *Malus:* integration of isozymes, DNA markers and morphological traits. Thesis. Cornell University, Ithaca, NY.

Hall, A. D. and M. B. Crane. 1933. Ile apple. Martin Hopkinson, London.

Hanna, W. W. and L. E. Towill. 1995. Long-term pollen storage. Plant Breeding Rev. 133:170-207.

Hauagge, R. and J. N. Cummins. 1991. Genetics of length of dormancy period in *Malus* vegetative buds. J. Am. Soc. Hort. Sci. 116:121-126.

Heilborn, 0. 1935. Reductive division, pollen lethality and polyploidy in apple. Acta Host. 11:129-184.

Hemmat, M., N. F. Weeden, A. G. Manganaris, and D. M. Lawson. 1994. Molecular marker linkage map of apple. J. Hered. 85:4-11.

Hemmat, M., N. F. Weeden, and S. K. Brown. 1995. Molecular markers for scab resistant (*Vf*) region in apple. HortScience 30:850 (Abstr. 914).

Henning, W. 1947. (Morphological, systematic and genetic investigations on the spades and species-hybrids of the genus Malus.) Züchter 17:289-349 (Plant Breeding Abstr. 19:376).

Hofer, M., and Y. Lespinasse. 1996. Haploidy in apples. In: In vitro haploid production in higher plants. Kluwer Academic, Dordrecht, Netherlands.

Höfte, H., and H. R. Whiteley. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis* Microbiol. Rev. 53:242-255.

Hunter, A. W. S. 1934. Tetraploidy in vegetative shoots of the apple induced by the use of colchicine. J. Hered. 45:15-16.

Hutchinson, A. 1967. Inheritance of stature, stooling, rooting ability, and other characteristics in progenies of Malling DC. p. 76-82. In: Annu. Rep. Hort. Inst. Ont. 1966.

James, D. J. 1991. *Agrobacterium*-mediated transformation of apple (*Malus pumila* Mill.). p. 213-226. In: M.R. Abuja (ad.). Woody plant biotechnology. Plenum, New York.

James, D. J., and A. M. Dandekar. 1991. Regeneration and transformation of apple (*Malus pumila* Mill.). p. 1-18. In: K. Lindsey (ad.). Plant tissue culture manual. Kluwer Academic, Dordrecht, Netherlands.

James, D. J., A. J. Passey, and D. C. Deeming. 1984. Adventitious embryogenesis and the in vitro culture of apple seed parts. J. Plant Physiol. 115:217-229.

James, D. J., A. J. Passey, and E. Rugini. 1988. Factors affecting high frequency plant regeneration from apple leaf tissues cultured in vitro. J. Plant Physiol. 132:148-154.

James, D. J., A. J. Passey, D. J. Barbara, and M. W. Bevan. 1989. Genetic transformation of apple (Malus psunila Mill.) using a disarmed Ti-binary vector. Plant Can Rep. 7:658661.

James, D. J., A. J. Passey, A. D. Webster, D. J. Barbara, P. Visa, A. M. Dandekar, and S. Uratsu. 1993a. Transgenic apples and strawberries: advance in transformation, introduction of genes for insect resistance and field studies of tissue cultured plants. Acta Hort. 336:179-194.

James, D. J., S. Uratsu, J. Chang, P. Negri, P. Viss, and A. M. Dandekar. 1993b. Acetosyringone and osmoprotectants like betaine or proline synergistically enhance *Agrobacterium*-mediated transformation of apple. Plant Cell Rep. 12:559-563.

James, D. J., A. J. Passey, and S. A. Baker. 1994. Stable gene expression in transgenic apple tree tissues and segregation of transgenes in the progeny: preliminary evidence. Euphytica 77:119-121.

Janick, J. 1974. The apple in Java. HortScience 9:13-15.

Janick, J. 1995. Breeding to biotechnology: Keeping the competitive edge. p. 22-28. In: Proc. 2nd Hort. Ind. Tech. Conf., 27-30 July, 1994 Austral. Soc. Hort. Sci.

Janse, J., J. J. Verhaegh, and A. P. M. den Mijs. 1994. Early selection for partial resistance to powdery mildew, *Podosphaera leucotricha* (Ell. at Ev.) Salm. in apple progenies. p. 13-15. In: H. Schmidt and M. Kellerhals (eds.). Progress in temperate fruit breeding. Kluwer Academic, Dordrecht, Netherlands.

Jeffers, S., and W. F. Wilcox. 1990. Phytophthora crown, collar and root rots. p. 43-45. In: A. L. Jones and H. S. Aldwinckle (eds.). Compendium of apple and pear diseases. APS Press, St. Paul, MN.

Jones, A. L., and H. S. Aldwinckle (eds.). 1990. Compendium of apple and pear diseases. APS Press, St. Paul, MN.

Karnatz, A. 1988. New apple cultivars after self-pollination. Acta Hort. 224:169-175.

Kellerhals, M., and B. Furrer. 1994. Approaches for breeding apples with durable disease resistance. Euphytica 77:31-35.

Kelsey, D. F., and S. K. Brown. 1992. 'McIntosh Wijick': a columnar mutation of 'McIntosh' apple proving useful in physiology and breeding research. Fruit Var. 1. 46:83-87.

Kemmer, E. 1953. On the primary and the fertile stages of apple orchards (in German). Züchter 23:122-127.

Keulemans, J., R. Eyssen, and G. Colda. 1994. Improvement of seed act and seed germination in apple. p. 225-228. In: H. Schmidt and M. Kellerhals (ads.). Progress in temperate fruit breeding. Kluwer Academic, Dordrecht, Netherlands.

King, G. 1995. Preliminary European apple genetic linkage map. p. 5. In: European Apple Issue 3.

Klein, L. G., R. D. Way, and R. C. Lamb. 1961. The inheritance of a lethal factor in apples. Proc. Am. Soc. Hort. Sci. 77:50-53.

Knight, R. L., and F. H. Alston. 1968. Sources of field immunity to mildew (*Podosphaera leucotricha*). Can. J. Genet. Cytol. 10:294-298.

Knight, R. L., and F. H. Alston. 1969. Developments in apple breeding. p. 125-132. In: Annu. Rep. E. Malling Res. Sta. 1968.

Knight, R. L., and F. H. Alston. 1972. Progress at East Malling in breeding for resistance to apple mildew *Podosphaera leucotricha.* p. 317-324. In: Annu. Rep. E. Malling Res. Sta., 1971.

Knight, R. L., J. B. Briggs, A. M. Massee, and H. M. Tydeman. 1962. The inheritance of resistance to woolly aphid, *Eriosoma lanigerium* (Hausmn.) in the apple. J. Hort. Sci. 37:207-218.

Koller, B., A. Lehmann, J. M. McDermot, and C. Gessler. 1993. Identification of apple cultivars using RAPD markers. Theor. Appl. Genet. 85:901-904.

Koller, B., L. Gianfranceschi, N. Seglias, J. McDermot, and C. Gessler. 1994. DNA markers linked to *Malus floribunda* 821 scab resistance. Plant Mol. Biol. 26:597-602.

Korban, S. S., and D. F. Dayton. 1983. Evaluation of *Malus* germplasm for sources of resistance to powdery mildew. HortScience 18:219-220.

Korban, S. S., and R. M. Skirvin. 1994. Nomenclature of the cultivated apple. HortScience 19:177-180.

Korban, S. S., and J. M. Swiader. 1994. Genetic and nutritional status in bitter pit-resistant and susceptible seedlings. J. Am. Soc. Hort. Sci. 109:428-432.

Korban, S. S.9 P. A. O'Connor, and A. Elobeidy. 1992. Effects of thidiazuron, napthaleneacetic acid, dark incubation and genotype on shoot organogenesis from *Malus* leaves. J. Hort. Sci. 76:341-349.

Krüger, J. 1994. Observations on different mildew sources used in apple breeding at Ahrensberg. p. 7-12. In: H. Schmidt and M. Kellerbals (ads.), Progress in temperate fruit breeding. Kluwer Academic, Dordrecht, Netherlands.

Lacey, C. N. D., and A. I. Cambell. 1987. Selection, stability and propagation of mutant apples. p. 351-361. In: A. J. Abbott and R. K. Atkin (eds.). Improving vegetatively propagated crops. Academic, New York.

Lambert, C., and D. Tepfer. 1992. Use of *Agrobacterium rhizogenes* to create transgenic apple trees having an altered organogenic response to hormones. Theor. Appl. Genet. 85:105-109.

Landry, B. S., R. Q. Li, W. Y. Cheung, and R. L. Granger. 1994. Phylogeny analysis of 25 apple rootstocks using RAPD markers and tactical gene tagging. Theor. Appl. Genet. 89:847-852.

Lapins, K. 1965. Compact mutants of apple induced by ionizing radiation. Can. J. Plant Sci. 45:117-124.

Lapins, K. 1969. Segregation of compact growth types in certain apple seedling progenies. Can. J. Plant Sci. 49:765-768.

Lapins, K. O. 1974. Spur type growth habit in 60 apple progenies. J. Am. Soc. Hort. Sci. 99:568-572.

Lapins, K. O. and R. Watkins. 1973. Genetics of compact growth. p. 136. In: Annu. Rep. E. Malling Res. Sta., 1972.

Laubscher, F. X., and Hurter, N. 1960. The chromosome numbers of seedling progeny from triploid apples. S. African. J. Sci. 3:31-39.

Lawson, D. M., M. Hemmat, and N. F. Weeden. 1995. The use of molecular markers to analyze the inheritance of morphological and developmental traits in apple. J. Am. Soc. Hort. Sci. 120:532-537.

Lespinasse, Y., and E. Chevreau. 1997. Progressi nel miglioramento genetico del melo: cloni autocompatibili e piante aploidi. Riv. Fruttic. Ortofloric. 69:25-29.

Lespinasse, Y., F. H. Alston, and R. Watkins. 1976. Cytological techniques for use in apple breeding. Ann. Appl. Biol. 82:349-353.

Lewis, D.. and M. B. Crane. 1938. Genetical studies in apples, II. J. Genet. 37:119-128.

Lockard, R. G., and G. W. Schneider. 1981. Stock and scion growth relationships and the dwarfing mechanism in apple. Hort. Rev. 3:315-375.

Luckwill, L. C. 1953. Virus diseases of fruit trees 4. Further observations on rubbery wood, chat fruit and mosaic in apples. p. 40-46. In: Rep. Long Ashton Res. Sta.

MacHardy, W. E. 1996. Apple scab: biology, epidemiology and management. APS, St. Paul, MN.

Maheswaran, G, M. Welander, J. F. Hutchinson, M. W. Graham, and D. Richards. 1992. Transformation of apple rootstock M.26 with *Agrobacterium tumefaciens.* J. Plant Physiol. 139:560-568.

Manganaris, A. G. 1989. Isozymes as genetic markers in apple breeding. Thesis, Wye College, University of London.

Manganaris, A. G., and F. H. Alston. 1988. The acid phosphatase gene, *ACP-1* and its linkage with the endopeptidase *ENP-1* and pale green lethal gene I in apple. Acta Hort. 224:177-184.

Manganaris, A. G., and F. H. Alston. 1989. Glutamate oxaloacetate transaminase isoenzymes in apple cultivars and rootstocks. J. Hort. Sci. 64:9-15.

Manganaris, A. G., F. H. Alston, N. F. Weeden, H.S. Aldwinckle, H. L. Gustafson, and S. K. Brown. 1994. Isozyme locus *Pgm-1* is tightly linked to a gene (*Vf*) for resistance in apple. J. Am. Soc. Hort. Sci. 119:1286-1288.

Marks, G. E. 1954. An acetocarmine-glycerol jelly for use in pollen fertility counts. Stain Technol. 29:277.

Markussen, T., J. Krüger, H. Schmidt, and F. Dunemann. 1995. Progress on mapping a gene for powdery mildew resistance in apple. p. 6. In: European Apple, Issue No. 3.

Marlow, G. C. and W. H. Loescher. 1994. Watercore. Plant Breeding Rev. 6:189-252

Martin, G. B. 1996. Molecular in cloning of plant disease resistance genes. p. 1-32. In: G. Stacey and N. Keen (eds.). Plant-microbe interactions, Vol 1. Chapman-Hall, New York.

Mauren M. M. F., R. M. Manshardt, D. Gonsalves, J. L. Slightom, and J. C. Sanford. 1992. Virus resistant papaya plants derived from tissues bombarded with the coat protein gene of papaya ringspot virus. Bio/Technology 10: 1466-1472.

McIntosh, D. L., and Lapins, K. 1966. Differences in susceptibility to apple powdery mildew observed in McIntosh clones after exposure to ionizing radiation. Can. J. Plant Sci. 46:619-623.

McIntosh, D. L., and F. C. Mellor. 1954. Crown rot of fruit trees in British Columbia, III: resistance trials on apple seedlings obtained from controlled crosses. Can. J. Agric. Sci. 34:539-541.

Menendez, R. A., F. E. Larsen, and R. Fritts. 1986. Fingerprinting apple cultivars by electrophoretic isozyme banding patterns. J. Environ. Hort. 4:101-107.

Michelmore, R. W. 1995a. Molecular manipulation of disease resistance genes. Annu. Rev. Phytopathol. 33:393-427.

Michelmore, R. W. 1995b. Isolation of disease resistance genes from crop plants. Current Opinion Biotechnol. 6:145-152.

Michelmore. R. W., I. Paran, and R. V. Kesseli. 1991. Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genome regions using segregating populations. Proc. Natl. Acad. Sci. (USA) 88:9828-9832.

Michelmore, R. W., R. V. Kesseli, D. M. Francis, I. Paran, M. G. Fortin, and C. H. Yang. 1992. Strategies for cloning plant disease resistance genes. p. 99-124. In: S. Guff, McPherson, and Bowles (eds.). Molecular plant pathology: a practical approach, Vol 2. IRL, Oxford, UK.

Mink, G. I., and J. R. Shay. 1962. Latent viruses in apple. Purdue Univ. Agric. Exp. Sta. Res. Bull. 756.

Mircetich, J. S. M., and G. T. Browne. 1989. *Phytophthora* root and crown rot of deciduous fruit trees: The influence of different rootstocks and soil moisture conditions on severity of the disease. Compact Fruit Tree 22:115-123.

Misic, P., and Z. Tesovic. 1970. Distribution of anthocyanin in the skin of apple varieties Jonathan, Red Delicious and their dark red mutants, 1. Sci. Agr. Res. Boegrad. 23:121-126.

Moore, M. H. 1960. Apple rootstocks susceptible to scab, mildew and canker for use in glasshouse and field experiments. Plant Path. 9:84-87.

Moore, R. C. 1940. A study of inheritance of susceptibility and resistance to apple cedar rust. Proc. Am. Soc. Hort. Sci. 37:242-244.

Morgan, J. and A. Richards. 1993. The book of apples. Ebury Press, London.

Mowry, J. B. 1964. Inheritance of susceptibility to *Gymnosporangium juniperi-virginianae*. Phytopathology 54:1363-1366.

Mowry, J. B., and D. F. Dayton. 1964. Inheritance of susceptibility to apple blotch. J. Hered. 55:129-132.

Murawski, H. 1955. Investigations of stages of apple seedlings as a basis for fruit research (in German). Arch. Gartenb. 3:255-273.

Murawski, H. 1967. A contribution to apple cultivation research, 10: results of cultivation of apple species with late leafing-out blooming (in German). Züchter 3:134-139.

Nonnecke, I. 1948. Fireblight resistance across the prairies in *Malus*. p. 31-32. In: Proc. W. Can. Soc. Hort. Sci.

Norelli, J. L., H. S. Aldwinckle, L. D. Beltran, and J. M. Jaynes. 1994. Transgenic 'Malling 26' [= 'M.7'] apple expressing the attacin E gene has increased resistance to *Erwinia amylovora.* Euphytica 77:123-128.

Nybom, N. 1959. On the inheritance of acidity in cultivated apples. Hereditas 45:332-350.

Opara, L. U., C. J. Studman, and N. H. Banks. 1996. Fruit skin splitting and cracking. Hort. Rev. 18:(in press)

Oppenheimer, C., and E. Slor, 1968. Breeding of apples for a sub-tropical climate, 2. Theor. Appl. Genet. 38:97-102.

O'Rourke, A. D. 1994. The world apple market. Food Product Press, New York.

Parisi, L., Y. Lespinasse, J. Guillaumes, and J. Kilger. 1993. A new race of *Venturia inaequalis* virulent to apples with resistance due to the *Vf* gene. Phytopathology 83:533-537.

Patat-Ochatt, E. M., S. J. Ochatt, and J. B. Power. 1988. Plant regeneration from protoplasts of apple rootstocks and scion varieties (*Malus xdomestica* Borkh.). J. Plant Physiol. 133:460-463.

Pratt, C. 1983. Somatic selection and chimeras. p. 172-185. In: J. N. Moore and J. Janick (eds.). Methods in fruit breeding. Purdue University Press, West Lafayette, IN.

Quamme, H. A., and C. Stushnoff. 1983. Resistance to environmental strew. p. 242-266. In: J. Janick and J. N. Moore (eds.). Methods in fruit breeding. Purdue University Press, West Lafayette, IN.

Redalen, G. 1988. Quality assessment of apple cultivars and selections. Acta Hort. 224:441-445.

Sadamori, S., T. Haniuda, S. Tsuchiya, and Y. Yoshida. 1964. (Studies on aberrant leaf in apple, 1: observations on the frequency of aberrant leaf in apples and their seedlings.) p. 1-7. In: Bull. Hort. Res. Sta. Morioka, Japan, Ser. C, No 2.

Sakai, A. 1994. Cryopreservation of apical meristems. Hort. Rev. 6:357-372.

Samimy, C., and J. N. Cummins. 1992. Distinguishing apple rootstocks by isozyme patterns. HortScience 27:829-831.

Sampson, D. R., and R. F. Cameron. 1965. Inheritance of bronze foliage, extra petals and pendulous habit in ornamental crab apples. Proc. Am. Soc. Hort. Sci. 96:717-722.

Save1jev, N. 1. 1989. Breeding material and genetic principles of breeding apple trees for frost and cold resistance. Acta Hort. 224:165-168.

Sax, K. 193 1. The origin and relationship of the Pomoideae. J. Arnold Arbor. 12:3-22.

Sax, K. 1959. The cytogenetics of facultative apomixis in *Maims* species. J. Arnold Arbor. 40:289-297.

Schmidt, H. 1964. A contribution to the cultivation of apomictic apple rootstocks, 1: cytogenetic and embryologic research (in German). Z. Pflanzenz. 52:27-102.

Schmidt, H. 1970. A contribution to the cultivation of apomictic apple rootstocks, 3: their affinity with cultured species in relation to virus content (in German). Z. Pflanzenz. 63:214-226.

Schmidt, H. 1988. Criteria and procedures for evaluating apomictic rootstocks for apple. HortScience 23:104-107.

Schmidt, H. 1994. Progress in combining mildew resistance from *Malus robusta* and *Malus zumi* with fruit quality. p. 3-6. In: H. Schmidt and M. Kellerhals (eds.). Progress in temperate fruit breeding. Kluwer Academic, Dordrecht, Netherlands.

Sewell, G. W. F., and Wilson, J. F. 1959. Resistance trials of some apple rootstock varieties to *Phytophthora cactorum* (L. and C.) Schroet. J. Hort. Sci. 34:51-58.

Shay, J. R., and L. F. Hough. 1952a. Evaluation of apple scab resistance in selections in *Malus.* Am. J. Bot. 39:289--297.

Shay, J. R., and L. F. Hough. 1952b. Inheritance of cedar rust resistance in apple. Phytopathology 42:19.

Shay, J. R., D. F. Dayton, and L. F. Hough. 1953. Apple scab resistance from a number of *Malus* species. Proc. Am. Soc. Hort. Sci. 62:348-356.

Shay, J. R., E. B. Williams, and J. Janick. 1962. Disease resistance in apple and pear. Proc. Am. Soc. Hort. Sci. 80:97-104.

Skirvin, R. M., M. Kouider, H. Joung, and S.S. Korban. 1986. Apple (*Malus domestica* Borkh.). p. 183-198. In: Y. P. S. Bajaj (ed.). Biotechnology in agriculture and forestry, 1: trees 1. Springer, Berlin.

Spinks, G. T. 1936. Apple breeding investigations, 1: results obtained from certain families of seedlings. p. 19-49. In: Rep. Long Ashton Res. Sta. 1936.

Sriskandarajah, S., P. B. Goodwin, and J. Speirs. 1994. Genetic transformation of the apple scion cultivar 'Delicious' via Agrobacterium tumefaciens. Plant Cell Tissue Organ Cult. 36:317-329.

Tancred, S. J., A. G. Zeppa, M. Cooper, and J. K. Stringer. 1995. Heritability and patterns of inheritance of the ripening date in apples. HortScience 30:325-328.

Tartarini, S. 1996. RAPD markers linked to the *Vf* gene for scab resistance in apple. Theor. Appl. Genet. (in press)

Thiele, I. 1950. Fruitfulness of maiden pome trees as a grower's project (in German). Züchter 26:241-243.

Thompson, A. H., and Batjer, L. P. 1950. The effect of boron in the germinating medium on pollen germination and pollen tube growth for several deciduous tree fruits. Proc. Am. Soc. Hort. Sci. 56:227-230.

Thompson, J. M., and 1. Taylor. 1971. Genetic susceptibility to *Glomerella* leaf blotch in apple. J. Hered. 62:303-306.

Tobutt, K. R. 1985. Breeding columnar apples at East Malling. Acta Hort. 159:63-68.

Tobutt, K. R. 1994. Combining apetalous parthenocarpy with columnar growth habit in apple. Euphytica 77:51-54.

Tukey, H. B. 1964. Dwarfed fruit trees. Cornell University Press, Ithaca, NY.

Tydeman, H. M. 1964. The relation between time of leaf break and of flowering in apple seedlings. p. 70-72. In: Rep. E. Malling Res. Sta., 1963.

Tydeman, H. M., and Alston, F. H. 1965. The influence of dwarfing stocks in shortening the juvenile phase of apple seedlings. In: p. 97-98. Rep. E. Malling Res. Sta., 1964.

Vail, P. V., J. S. Tebbets, D. F. Hoffmann, and A. M. Dandekar. 1991. Response of production and postharvest walnut pests to *Bacillus thuringiensis* insecticidal crystal protein fragments. Biol. Control 1:329-333.

Vavilov, N. I. 1930. Wild progenitors of the fruit trees of Turkistan and the Caucasus and the problems of the origin of fruit trees. p. 271-286. In: Rep. 9th Int. Hort. Congr. Royal Hort. Soc., London.

Vinterhalter, D. V., and D. T. James. 1983. The use of peroxidase polymorphism in the identification of apple scion cultivars. Sci. Hort. 18:253-261.

Vinterhalter, D. V., and D. T. James. 1986. The use of peroxidase polymorphism in the identification of Malling and Malling Merton apple rootstocks. J. Hort. Sci. 110:509-515.

Visser, T. 1951. Floral biology and crossing technique in apples and pears. Meded. Dir. Tuinb. 14:707-726.

Visser, T. 1964. Juvenile phase and growth of apple and pear seedlings. Euphytica 13:119-129.

Visser, T. 1970. Environmental and genetic factors influencing the juvenile period in apple. p. 101-115. In: Proc. Angers Fruit Breeding. Symp.

Visser, T., J. J. Verhaegh, and D. P. de Vries. 1971. Preselection of compact mutants induced by X-ray treatment in apple and pear. Euphytica 20:153-207.

Wallin, A., and L. Johansson. 1989. Plant regeneration from leaf mesophyll protoplasts of in vitro cultured shoots of a columnar apple. J. Plant Physiol. 135:565-570.

Watkins, R., and R. A. Smith (eds.). 1982. Descriptor list for apple (*Malus*). Int. Board Plant Genetic Resources, Rome.

Watkins, R., and L. P. S. Spangelo. 1970. Components of genetic variance for plant survival and vigor of apple trees. Theor. Appl. Genet. 40:195-203.

Watkins, R., and J. M. Werts. 1971. Preselection for *Phytophthora cactorum* (Leb. and Conn.) Schroet. resistance in apple seedlings. Ann. Appl. Biol. 67:153-156.

Way, R. D. 1971. Hastening the fruiting of apple seedlings. J. Am. Soc. Hort. Sci. 96:384-389.

Way. R. D., H. S. Aldwinckle, R. C. Lamb, A. Rejman, S. Sansavini, T. Shen, R. Watkins, M. M. Westwood, and Y. Yoshida. 1990. Apples (*Malus*). p.1-62. In: J. N. Moore and J. R. Ballington Jr. (eds.). Genetic resources of temperate fruit and nut. Int. Soc. Hort. Sci., Wageningen (Acta Hort. 290).

Weeden, N. F. 1989. Applications of isozymes; in plant breeding. Plant Breeding Rev. 6:1154.

Weeden, N. F., and R. C. Lamb. 1985. Identification of apple cultivars by isozyme phenotypes. J. Am. Soc. Hort. Sci. 110:509-515.

Weeden, N. F., and R. C. Lamb. 1987. Genetics and linkage analysis of 19 isozyme loci in apple. J. Am. Soc. Hort, Sci. 112:865-872.

Weeden, N. F., G. M Timmerman, M. Hemmat, B. E. Kneen, and M. A. Lodhi. 1992. inheritance and reliability of RAPD markers. p. 12-17. In: Application of RAPD technology to plant breeding. Joint Plant Breeding Symp. Series. Crop Sci. Soc Am./Am. Soc. Hort. Sci./Am. Genet. Assoc.

Weeden, N. F., M. Hemmat, D. M. Lawson, M. Lodhi, R. L. Bell, A. G. Manganaris, B. Reish, S. K. Brown, and G. Ye. 1994. Development and application of the molecular marker linkage maps in woody crops. p. 269-273. In: H. Schmidt and M. Kellerhals (eds.). Progress in temperate fruit breeding. Kluwer Academic, Dordrecht, Netherlands.

Welander, M. 1988. Plant regeneration from leaf and stem segments of shoots raised in vitro from mature apple trees. J. Plant Physiol. 132:738-744.

Welander, M., and G. Maheswaran. 1991. Regeneration and transformation experiments in apple. p. 237-246. In: M. R. Ahuja (ed.). Woody plant biotechnology. Plenum, New York.

Weller, D. L., and J. F. Costante. 1986. Peroxidase zymograms of 16 apple rootstocks. Can. J. Plant Sci. 66:647-652.

Welsh, J., and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 19:303-306.

Wilcox, A. N., and E. Angelo. 1936. Apple breeding studies, 1: fruit color. Proc. Am. Soc. Hort. Sci. 33:108-113.

Wilcox, A. N., and E. Angelo. 1937. Apple breeding studies, 2: fruit shape. Proc. Am. Soc. Hort. Sci. 34:9-12.

Williams, E. B., and J. Kuc. 1969. Resistance in *Malus* to *Venturia inaequalis*. Annu. Rev. Phytopathol. 7:223-246.

Williams, J. G. K., A. R, Kubelik, K. J. Livak, and J. A. Rafalski. 1990. DNA polymorphisms amplified as arbitrary primers are useful genetic markers. Nucleic Acids Res. 18:6531-6535.

Williams, W. 1958. Dept. Plant Breeding. p. 6-13. In: Annu. Rep. John Innes Hort. Inst. 1957.

Williams, W. 1959. Selection of parents and family, size in the breeding of top fruits. p. 211-213. In: Rep. 2nd Congr. Eucarpia.

Williams, W., and Brown, A. G. 1956. Genetic response to selection in cultivated plants: gene frequencies in *Prunus avium*. Heredity 10:237-245.

Wormald, H. 1955. Diseases of fruit and hops. Crosby Lockwood, London.

Yahia, E. M. 1994. Apple flavor. Hort. Rev. 16:197-234.

Yang, H., and J. Kroger. 1994. Identification of an RAPD marker linked to the *Vf* gene for scab resistance in apples. Plant Breeding 112:323-329.

Yao, J. L., D. Cohen, R. Atkinson, K. Richardson, and B. Morris. 1995. Regeneration of transgenic plants from the commercial apple cultivar 'Royal Gala'. Plant Cell Rep. 14:407-412.

Zhang, Y. X., and Y. Lespinasse. 1988. Culture in vitro d'ovules non fecondes d'embryons preleve 8 jours apres pollinisation chez le pommier cultive (*Malus xdomestica* Borkh.). Agronomie 8:837-842.

Zhang, Y. X., Y. Lespinasse, and E. Chevreau. 1988a. Obtention de plantes haploides de pommier (*Malus xdomestica* Borkh.) issues de parthenogenese induite in situ par du pollen irradie et culture in vitro des pepins immature. C. R. Acad. Sci. Paris 307:451-457.

Zhang, Y. X., Y. Lespinasse, and G. Salesses. 1988b. Mise en evidence de quelques anomalies meiotiques conduisant a la formation de gametes males non reduits chez le pommier cultive (*Malus xdomestica* Borkh.). Cytologia 53:749-755.

Zhang, Y. X., G. Salesses, and Y. Lespinasse. 1988c. Etude cylogenetique de quelques clones diploides et polyploides de pommier (*Malus xdomestica* Borkh.). Considerations quant a l'origine du pommier. Cytologia 53:739-748.

Zimmerman, R. H. 1971. Flowering in crab apple seedlings: methods of shortening the juvenile phase. J. Am. Soc. Hort. Sci. 96:404 411.

Zimmerman, R. H. 1984. Apple. p. 369-395. In: W. R. Sharp, D. A. Evans, P. V. Ammirato, and Y. Yamada (eds.). Handbook of plant cell culture, Vol. 2. crop species. Macmillian, New York.

Zimmerman, R. H. 1991. Micropropagation of temperate zone fruit and nut crops. p. 231-246. In: P. C. DeBergh and R. H. Zimmerman (eds.). Micropropagation. Kluwer, Netherlands.

Zimmerman, R. H., and 1. Fordham. 1985. Simplified method for rooting apple cultivars in vitro. J. Am. Soc. Hort. Sci. 110:34-38.