The USDA Pear Breeding Program II. Seedling Evaluation

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Abstract

A detailed description is presented of the collection and handling of pear seed from controlled pollinations, and the planting, inoculation, evaluation and culture of seedlings in the greenhouse and in the field. During the past decade, data on fire blight resistance, fruit quality, juvenility, and male sterility have been collected on approximately 30,000 seedlings planted at Beltsville, Maryland, and Wooster, Ohio. Observations were also made on overall resistance to Fabraea leaf spot, pear psylla and leaf scorch. Numerous selections are presently under observation. All data are computerized and utilized in the genetic interpretation of specific characters as well as for the decision making for selections and choosing parents in the breeding program.

This is Part II of a continuation of the report on different phases of the pear breeding program of the United States Department of Agriculture (5). The program is designed to allow the development of new cultivars which have combined resistance to various diseases and insects and to provide genetic information on the inheritance patterns of various characters. In the first report, we described pollen collection, pollination, the number of seed obtained and the number of pollinations required to obtain the desired number of seeds. Here, we describe seed handling, planting, screening against diseases, field practices to decrease the juvenile period, data collection and various scoring systems.

Seed Collection—Fruit from controlled crosses are collected from the identified branches in late August or early September and are placed in storage at 32-34°F (0-1°C). Usually in October, seeds are extracted by cutting the fruit at its widest diameter. The partially divided fruit is twisted apart, and the seeds are collected, washed with running tap water for 15 minutes using a Buchner funnel. Water from the tap flows up through the funnel and the lighter non-seed particles are washed away. The seeds are surface sterilized by placing them in diluted merthiolate (1:2000) for 5 minutes, then rewashed with running tap water through the funnel for 15 minutes. Washed seeds are dipped in a suspension of 6 gm/liter of 50% benomyl for prevention of mold and are placed on 2 moist filter paper disks in the bottom of a petri dish (100 x 15 mm) and then covered with the top of the dish. The seeds are stratified in this moist condition for 90 days at 34°F (1°C). After this time, seeds will germinate at a room temperature of 72°F (22°C).

Planting—Seedlings from controlled pollinations are raised in the greenhouse for disease evaluation and/or subsequent planting in the field. Planting is usually done in January. By this time, the seeds are stratified and can be planted. Early planting is desirable because the plants will then

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We acknowledge the assistance by Mr. W. A. Zook, who through the years has performed all phases of seed collection and planting, seedling inoculation and field transplanting, and collection of fruit samples, and has also provided assistance with general tree maintenance and collection of field data.

We also acknowledge the input in past years on several phases of the breeding program, in particular those by Dr. H. J. Brooks (National Program Staff), Mr. W. A. Oitto (retired), and numerous technicians and students who assisted in all phases of the work.

Fig. 1. Greenhouse procedures in the USDA pear breeding program:
A—young seedlings in 6.0 cm peat pots growing under 1000 watt multi-vapor lamps.

have sufficient time to grow before inoculation with the blight organism; if inoculated in April, they can be planted in the field in late May. Late May planting allows the plants a full season of growth in the field which shortens their juvenile period and speeds the breeding program. Thus, late May planting necessitates seeding in early January.

Seeds are planted in peat pots (6 cm) with 12 pots to a plastic tray; the pots are filled with a commercial mixture of sterile, shredded sphagnum peat moss and a horticultural grade vermiculite fortified with fertilizer. With these trays, about 360 plants can be grown on each yard^2 (m^2) of greenhouse bench. Germinated seeds are planted individually in each pot with their radicles downward. Greenhouse temperature is maintained at 77°F

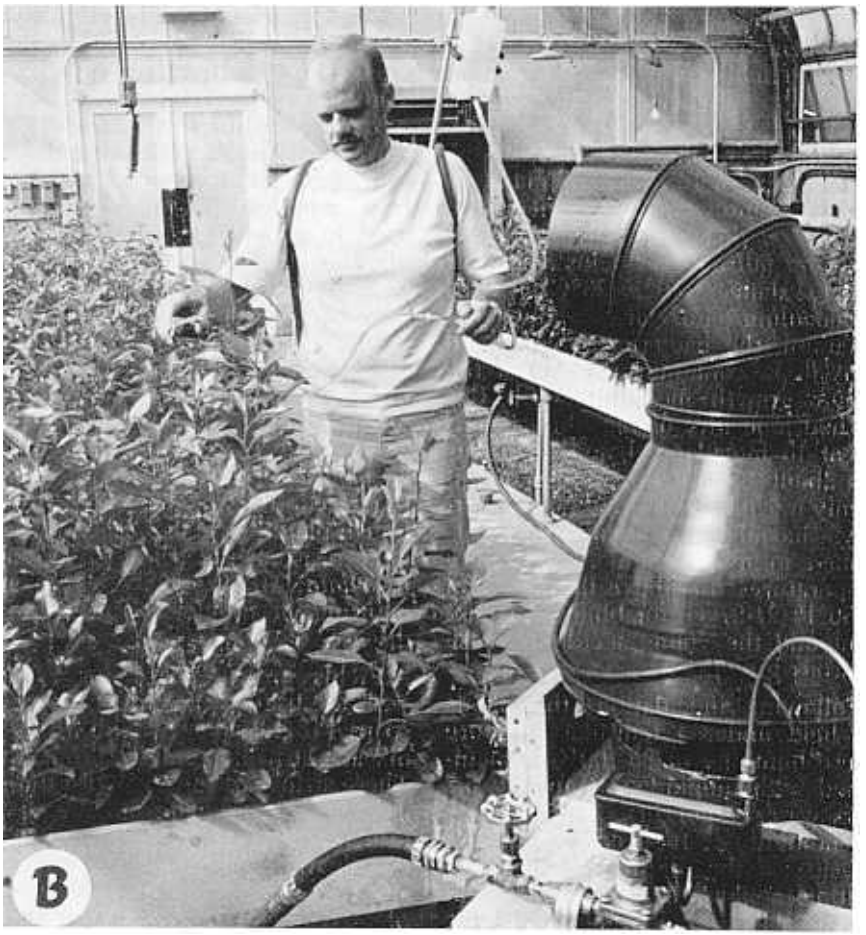

Fig. 1. B—inoculation of succulent shoot tips of 3-month-old seedlings with the mass inoculator attached by plastic hose to bottle of inoculum carried on back pack of aluminum tubing. Humidifier in foreground supplies vapor needed for optimum conditioning for infection.

(25°C) with overhead lights being used to increase the growing time during the overcast days of January and February (Figure 1-A). The lamps are 1000 watt multivapor units, with the base of the covers hanging approximately 5 ft. (1.5 m) above the bottom of the bench. To prevent damping off during the first 30 days, the young seedlings are watered weekly with an aqueous suspension of ferbam at a dose of 1 level teaspoon/gallon of water (2.5g/3.8 liters). A general greenhouse spray program is followed for insect and fungal control. Liquid fertilizer (20-20-20) is routinely applied every 2 weeks to maintain succulent growth of the seedlings. After about 3 months, the plants reach a height of 8-16 inches (20-40 cm) and are ready for inoculation with the blight pathogen.

Inoculation—Most pear seedlings are screened for their resistance to fire blight by inoculating succulent shoot tips with the bacterium *Erwinia amylovora* (Figure 1-B). A Special device is used for this purpose (2). Usually, a suspension of 10^6 cells/ml, made up from one culture of a virulent isolate of *E. amylovora,* grown for 24 hours on nutrient-yeast-dextrose agar (NYDA) slants at 79°F (26°C), is used as inoculum. For large volumes of inoculum, the bacteria are grown in Roux flasks containing 250 ml of the NYDA growth medium. With this method, one liter of inoculum is sufficient to inoculate about 300 plants and about 4500 seedlings can be uniformly inoculated per day (4). Preferably, inoculations should be performed in mid to late April before temperatures rise above the optimum 70-80°F (21-27°C) for blight development and the young seedlings cease vigorous growth.

Following inoculation, the plants are held under a tent-like structure where humidity of 85-100% is maintained with humidifiers (capacity 5.7 liters water vapor/hr). After 5 days, the humidity is reduced by opening the side of the plastic structure and wetting the floors and benches several times a day. Blight usually kills the seedling or it stops spreading in about 30 days. Six weeks after inoculation, total height and the blighted portion of each plant are measured. Only seedlings which do not blight more than about 25 percent are transplanted to the field.

Some progenies are not inoculated. They are planted in the field for use in genetic studies. Inoculation would preclude their involvement in these studies in some cases because of small populations.

Field Practices—At Beltsville, seedlings are planted in the field during the third week of May. They may be planted in one of four areas, depending upon the purpose of the specific cross and upon numbers of seedlings available. The first area is for unscreened seedlings which are grown with adequate insecticide and fungicide spray control to insure quality fruit samples. These seedlings are used in genetic studies. The second planting is for seedlings that have been screened in the greenhouse and have been found to be resistant to fire blight. These are also protected against other diseases and insects. The third planting is used to evaluate seedlings from special crosses for resistance to the pear psylla (*Psylla pyricola* Foerster), omitting the insecticide control for this insect. The fourth planting is established to determine resistance to leaf spot (*Fabraea maculata* Atk.) of given progenies, omitting the fungicide control for this disease.

Fields are prepared for planting with the use of a rototiller, preparing strips 4 ft (1.2 m) wide with the center of each strip being 16 ft (5 m) apart. These strips are sprayed with the preemergence herbicide DCPA at the rate of 8 lb/acre (1.5 kg/ha) to kill germinating weed seeds. During 1965-74, seedlings at Beltsville were planted by spacing them 4 ft (1.2 m) in the row, in 3 ft (1 m) double rows 16 ft (5 m) apart. They are irrigated the same day of planting to insure successful transplanting. In the first year, the irrigation system is used through the drier months of June, July, and August to establish a good root system.

In Ohio, seedlings are planted in the fall 4 ft (1.2 m) apart in a single row with 16 ft (5 m) between rows. This means that the plants have to be kept in the nursery an entire season, which in turn lengthens their juvenile period and delays fruit production. For the first two years seedlings are

cultivated, after which herbicides are sprayed on the tree rows. Every year in the spring, an application of simazine at 5 lb/50 gal water (2.3 kg/190 l) is made prior to the beginning of weed growth. Paraquat at 1.2 l/50 gal water (1.2 l/190 l) is sprayed later in the growing season to kill weeds in the rows. The grass growing between the strips is mowed periodically when needed.

In March, urea is applied at the rate of about ⅛ lb (57 grams) for each year of tree growth. In late April or early May, 10-10-10 fertilizer is applied at the same rate. Fertilizer is not spread on the grass strips. Trees are fertilized to maintain fast growth which may support susceptibility to fire blight infection, and also decreases the period of juvenility.

Fire blight is not controlled by spraying or pruning since a natural spread of the disease is desired in the breeding program to eliminate the susceptible trees not detected in the greenhouse screening program.

Seedling trees are maintained at Beltsville for 8 years and at Wooster for 10 years and are observed annually for tree and fruit characteristics. Trees which have possible combined characteristics of desirable growth habits, fire blight, leaf spot, or psylla resistance, and/or superior fruit quality are propagated for observation in a separate test planting (see Part IV in this series).

Data collection—Data are taken annually in the field on all seedlings regarding number of flower buds, male sterility, and degree of fire blight resistance. Data on leaf spot and pear psylla resistance are taken on seedlings with other superior characters and on those in two special plantings established to study these two particular problems. Data are recorded on a special form (Figure 2) from

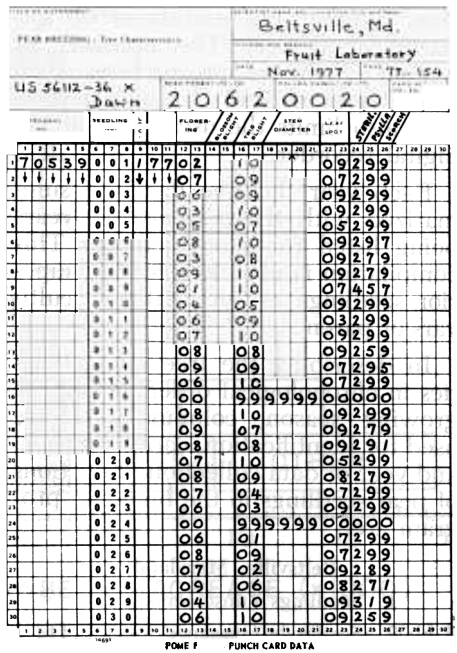

Fig. 2. Data record sheet used for horticultural and pathological tree characteristics of pear seedlings.

which they are keypunched, proofed and stored on tape for future use.

A master copy of a given planting is maintained for the life of the planting. Missing tree codes and male sterility data are posted on the master each year, from which copies can be made for next year's data. This eliminates reading male sterility more than once and increases accuracy of the records, especially when there are missing trees in the row.

Recently, flower bud data at Beltsville have been recorded on a portable digital tape recorder. Direct transfer of data from the cassette tape to 9-track magnetic tape eliminates keypunching and proofing. This simplifies data collection but the digital data display is difficult to see outdoors,

thus limiting the usefulness of this particular device. The necessity of having a record of missing trees when recording fire blight and male sterility data also limits the usefulness of the digital recorder.

Each year the tree data are summarized with the data for all years being listed for each seedling. This compilation is used in selecting superior seedlings as potential new cultivars or parents.

In describing the tree characteristics in chronological order during the growing season, columns 1-11 are used for identification, coded location, and year of data collection. Locations of pear seedlings in the breeding program are:

Beltsville, Maryland

1. Seedlings (unscreened)
2. Seedlings (screened for blight)
3. Propagated trees

Wooster, Ohio

4. Seedlings (unscreened)
5. Seedlings (screened for blight)
6. Propagated trees

Flowering data (columns 12-13) are recorded in early spring (before bloom), by using the following numerical scale:

Score	No. of flower buds
9	over 500
8	251-500
7	101-250
6	51-100
5	26-50
4	11-25
3	6-10
2	1-5
1	0 (tree alive)
0	tree dead or missing

The score is established by estimating the number of flower buds per tree (6).

Data on male sterility are collected toward the end of the bloom season and are recorded in column 24. Usually, pear flowers completely devoid of any fertile anthers are small and cup-shaped (Figure 3-A). Immediately following spring pollinations, all seedling trees not previously rated are observed for the presence or absence of pollen using the following codes:

1 = sterile, no pollen
2 = fertile, pollen present
3 = blossom time missed
4 = no blooms

In all flowering data, a missing or dead tree is indicated with a zero. Male sterility is quite prevalent in pear cultivars such as Waite and Magness. It appears to be cytoplasmic in nature, is transmitted to its seedling progenies, and should be avoided in the breeding program (1). Magness trees are visited infrequently by bees and they are usually unproductive, even if planted with sufficient pollinizers.

Toward the end of the growing season (July-August), evaluations are made for susceptibility to leaf spot, psylla, and scorch. In order to rate seedlings with accuracy, scoring must be done at a certain time to distinguish between leaf spot and psylla. Leaf spot produces gray to brown-black spots, sometimes with raised pustules, which later coalesce to form large necrotic areas (Figure 3-B). Defoliation progresses from the lower limbs toward the top of the tree. Pear psylla nymphs suck juices from the leaves and secrete a honeydew excrement that glistens and runs down over the fruit and foliage. A mold-like fungus grows on the honeydew, resulting in black blotches on fruit and foliage (Figure 3-C). In areas where both problems occur, field readings for leaf spot should be made earlier (about early to mid August) than

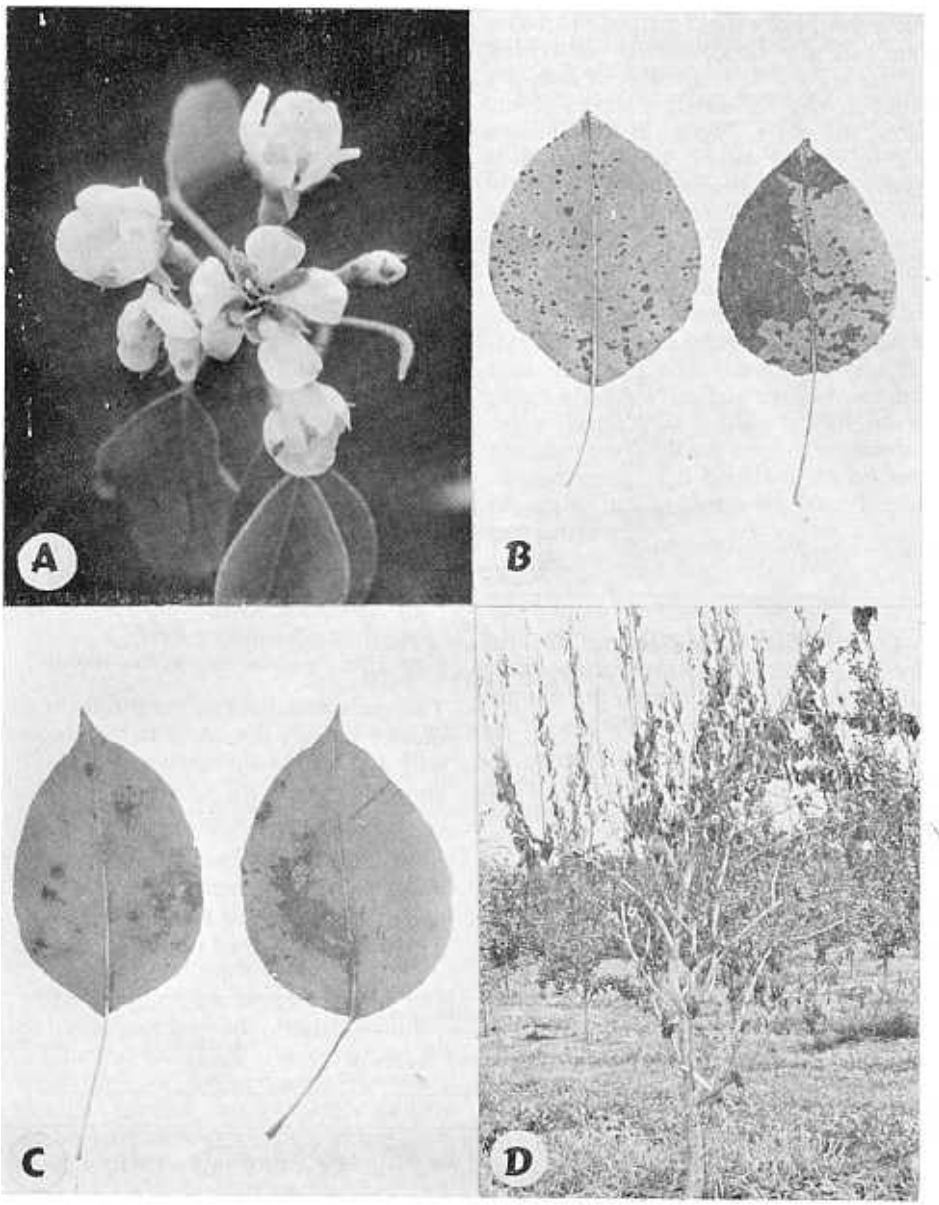

Fig. 3. Four characteristics of pear seedlings and selections under study in the USDA pear breeding program:

A—male sterility of blossoms;
B—Fabraea leaf spot;
C—pear psylla; and
D—fire blight.

those for psylla (September). Defoliation can also be caused by scorching of the leaves. It is a result of hot, dry weather that causes the leaves of the more susceptible trees to turn brown and curl. However, this disorder is easily distinguishable from leaf spot and psylla. The numerical scoring system for all three problems is as follows:

Score	% Defoliation
9	0
8	1-6
7	7-12
6	13-25
5	26-50
4	51-75
3	76-88
2	89-99
1	100

On the data sheet, scores for leaf spot are recorded separately from those for psylla and scorch in columns 22-23, 25, and 26, respectively.

Data on fire blight are usually recorded in mid or late October, when fall leaf coloration and early defoliation simplify observation of cankers and blighted branches with clinging black leaves (Figure 3-D). The following numerical scale is used for evaluating fire blight:

This scoring system is based on 1) the number of branches infected, 2) the age of wood into which the blight organism has penetrated, and 3) the overall percentage of tree blighted (3). Due to the general absence of blossom blight in Beltsville, no data are recorded on this.

At the time the fire blight data are taken, a system of code numbers is used to designate very small, unthrifty and dead or missing trees for reasons other than fire blight. The code numbers are as follows:

21 Mehanical damage
22 Frost damage, heaving, winter injury
23 Tree removed
24 Unknown
25 Sprouts only
26 Rough bark
27 Very weak and unusually small tree
28 Herbicide injury
30 Transplanted tree dead
40 New growth—reactivates record

The code numbers are entered on the master record file so that this record will appear on successive years. Obviously, trees with scores 25 through 27 are ignored for blight readings.

The first year that the trees come into production, fruit which best represent the seedling tree are picked as a sample. Details of these and general

Blight rating	Blight Infection into				Portion of tree blighted
	Current season wood	2-year-old wood	3-year-old older wood	Scaffold limbs of trunk	
	no.	no.	no.		%
	none	——	——	——	0
	few	——	——	——	1 - 3
	several	few	——	——	4 - 6
	many	several	few	upper ⅛	7 - 12
	——	many	several	upper ¼	13 - 25
	——	——	many	upper ½	26 - 50
	——	——	——	lower ½	51 - 75
	——	——	——	lower ¼	76 - 88
	——	——	——	base	89 - 99
	——	——	——	all	100

procedures in fruit picking, storage and evaluation will be discussed in Part III of this series.

Discussion

Between 1962 and 1971, approximately 2500 seedlings were planted annually at Beltsville and, since 1966, about 1200 at Wooster, Ohio. The initial plan was to grow the trees on a 10-year rotation cycle. With better growth and reduction of the juvenile period, this cycle has been reduced to 8 years at Beltsville. This schedule has been slightly modified recently by leaving the seedlings one extra year without psylla control sprays. Records are taken on defoliation as an indication of psylla resistance or susceptibility. As soon as superior seedlings can be identified, selections are tagged, propagated and more detailed tree data are collected. For additional information on tree and fruit characteristics of selections made in the pear breeding program, see Part IV of this series.

Spring frost damage in Maryland and Ohio in recent years has seriously reduced seed production from hybridization and subsequent seedling planting. Good cooperation with pear breeding programs at Rutgers University in New Jersey, the New York Agricultural Experiment Station at Geneva, the Canada Department of Agriculture at Harrow, Ontario, the East Malling Research Station in England, and the National Research Institute at Angers, France, have provided us with extra seed lots in the off years. Such cooperative efforts are extremely valuable in our pear breeding program.

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Self-Unfruitfulness of 'Anna' Apple[1]

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Additional Index Words

Malus, Pollination.

Abstract

Fresh pollen of 'Anna', 'Dorsett Golden', FL 1W-22 and ether-killed 'Dorsett Golden' mixed with 'Anna' about 1:1 was used to pollinate approx 650 flowers of 'Anna' apple to determine self-fruitfulness. 'Anna' is not self-fruitful. 'Dorsett Golden' was cross-fertile with 'Anna' and is recommended as a pollenizer because it matches 'Anna's' blossom period.

'Anna' apple originated in Israel and was introduced by Abba Stein in 1963.

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