THE FRENCH WALNUT BREEDING PROGRAM: Trip Notes

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ABSTRACT

This is an informal report on the French breeding program of E. Germain based on a visit I made to France in 1992. My purpose in describing the program is to allow those in my program as well as cooperators to evaluate some of the procedures used there and to determine how to use the information to improve our program. The French breeding program is very active; Germain has three full time technicians. It includes both variety and rootstock development. The variety work is based on the Serr/Forde model and three new varieties will be released soon. They have been evaluated for response to blight under controlled conditions and should be more resistant than other materials. They will also be evaluated for response to cold in growth chambers. The rootstock selection work is similar to ours and uses backcrosses to eliminate hypersensitivity to blackline. Clonal propagation is a challenge for both of us, although Germain is more confident in micropropagation than we are, partly because of a semi-private organization that multiplies shoots for the nurseries. Overall the program is successful and is aimed at substantially increasing French walnut production.

OBJECTIVE

The purpose of this trip (May 9-15, 1992) was to review our walnut descriptor list with the French walnut breeder, Eric Germain, who works for INRA (Institut National de la Recherche Agronomique) at the Grand Ferrade Research Station near Bordeaux. The trip was funded by IBPGR (International Bureau of Plant Genetic Resources) and my responsibility was to provide them with an internationally acceptable descriptor list for walnuts. They plan to publish the list as soon as possible.

Reviewing the list took about 2 1/2 days, so in the remaining time Germain and I visited his field plots, discussed his program, and visited a nurseryman, Jean Couillie, in Larche, about a half day’s drive away. Bob Driver had told me about this man who had been clonally propagating chestnuts and walnuts.

This informal report is my attempt to record what I learned in conversations with Germain. Our breeding program should consider some of the techniques he uses and decide if they would make us more efficient.

THE PROGRAM

Breeding Early efforts in walnut improvement in France were aimed at scouring the French countryside for new varieties, testing the many different clones of Franquette and Parisienne, and introducing and evaluating germplasm. The U.S. varieties were found more precocious and higher yielding than any varieties they found in France, but the US varieties leaf out too early for France. Germain finally concluded that breeding was going to be the only means of finding a good variety for France and he has been actively pursuing this goal with his three full time technicians.
Germain made his first crosses in 1977, shortly before Harold Forde retired from breeding here. His goals were late leafing (Franquette-time), lateral fruitfulness, high quality nuts and some tolerance to blight. Recently he has separated selections aimed for the shelled market from selections aimed for inshell trade. At first Germain only used Franquette as the female and Payne and Ashley as males. Later he also used Marbot, Grandjean, Grosvert-Verdot and Adams 10 as females; and Serr, Chico, Lara and Pedro as males. Recently he has been using Chandler and finds it the best parent for quality. His goal is to obtain 100 seedlings that leaf out no more than two weeks before Franquette from each cross, which means he might have to make the same cross over several years. (Note we aim for about 50 seedlings/cross.)

To make crosses he collects pollen, holds it 4 hours over calcium chloride to dry to 7-8%, and stores it in the freezer, -25 to -30°C. Before using, he tests pollen viability by a FCR reaction (Florecin acetate) and finds this test as good and much quicker than the germination tests. He has tested the bags we use but although he finds them superior, because of the cost, he continues to use his cloth/paper combination bags. He achieves 25-60% set. Since his bags have no window he has to remove the bag to pollinate the flowers. He knows that there is a possibility of outside pollen contamination but he has never had a nut set in his controls (treated the same but no pollen applied) and isozyme analysis of his selections suggested that only one was in error (1/40, I think).

He ties net bags around the developing nuts just like we do (we learned that from him) and after harvest stores them dry and cold. For germination, he slightly cracks the nuts and soaks them in GA3 (50ppm) for one day, drains them for one day and then germinates them in the greenhouse. It takes about two weeks. His germination is not great (50%) and he thinks he might be selecting against those that need a long stratification time.

He keeps the seedlings in the greenhouse for about 1 1/2 months and then plants them in the nursery under cloth in early May. At the end of one year they are about 30-40 cm tall. The following spring he evaluates leafing date and at the end of that growing season he measures height. They should be about 1 1/2 meters tall. He then eliminates any that are more than 2 weeks earlier than Franquette or are not vigorous. He thinks that vigor is essential in precocious varieties so that they have some size before fruiting. Varieties like Howard and Vina he considers too weak.

After the two growing seasons in the nursery, the selected seedlings (about 40-50% of the nursery trees) are transplanted to a selection block at 6 X 4 meters. He transplants them in the order of leafing to make evaluation easier. The selection block always has some Ronde de Montignac grafted in it as a precocious source of pollen.

The seedling selections usually flower in their third year in the selection block but he does not begin to evaluate them until their 4th year (6th year from seed). He evaluates them for 4 years. After 2 years evaluation he begins to make selections and by the end of the third year he is ready to graft promising selections into a clonal selection block. Thus, the best go into the clonal selection block about 8 to 9 years from seed, after 3-4 years evaluation.
Of his 24-25 different crosses, he has 2000 seedlings in the seedling selection block; evaluation is complete on about 1000 of them. He has selected 42-43 hybrids, as he calls them, as advanced selections. Each advanced selection is not only grafted into his clonal selection block but also into two CTIFL field trials where each is grafted onto three mature trees per location. The CTIFL trials are overseen by Prunet and Charlot.

Germain expects to protect (by UPOV) and release 4 cultivars in 1996. These are 94-11, 94-12, 94-104, and 102-03. All are of the cross Franquette by Lara except the latter which is Grosvert-Verdot by Lara. He has had a meeting with the nursery growers and if they want to be licensed they must grow 50 trees of each as scionwood mother trees. They also must agree to remove all trees of any clone that Germain decides not to protect (=patent). About 10 nurseries have started to propagate the 4 potential cultivars. (This seemed like plans for some very big plantings beginning in 1996.)

**Evaluation** (Here I will just concentrate on what he does differently from us.) First he culls about 50% of the seedlings at age two in the nursery, a cull based solely on vigor and leafing date. This early selection has made it difficult for him to do any genetic studies which he regrets and says in the future he plans to make fewer crosses and do more research.

In the seedling selection block, where evaluations start in the 4th year from transplant, he evaluates lateral fruitfulness, shoot blight, form and vigor in the winter. Lateral fruitfulness he classifies as US, European, or intermediate like Hartley. Form is classified as erect, semi-erect or spreading; semi-spreading is also used. Vigor is calculated from the circumference measured 40 cm from the soil in ungrafted trees or 20 cm from the graft union in grafted trees. Usually he measures trees at the end of the second growing season in the nursery, and in their 4th and 7th years in the seedling selection block, that is before flowering and several years after flowering.

In the spring he walks through the trees only about 6 times. Each time he notes the stage of development (see Le Noyer) of leaves and male and female flowers. From this he can classify the phenology of tree on a 1-9 scale from very early to very late. He can also come very close to estimating the actual dates of leafing, and peak male and female bloom. His criteria for noting leafing date is actually earlier than ours and is based on when the buds turn green (i.e. when you can see the green of the leaf in the bud). He chose this stage because it is when the buds become susceptible to cold injury. (I agreed that Germain's system was more useful than ours, especially where spring frosts are a problem.)

Blight is scored for leaves in the beginning of July and at the end of July. The first notation for blight on fruits is also at the end of July and again in mid-August. Shoots are scored in the winter. There is some recent work being done by Charlot that suggests that blight is associated with soil type and by Jay-Allemand that it is associated with juglone or naptha-quinone content of leaves.

In the fall he evaluates harvest date like we do, and the crack out is also similar but is based on 20 nuts and he has an added class for kernel color of extra light (both changes I included in the descriptor list). Many of the
details on shell tip shape and thickness of the suture are only used for
variety description for UPOV protection.

Germain is now testing his advanced selections for response to blight, using a
fog system and artificial inoculations. This trial is right outside his
window. The fog goes on for 20 seconds every 18 minutes, such that the leaves
are wet but not dripping (so the inoculum doesn't wash off) for 8-10 minutes
and dry for 8-10 minutes. He can begin reading the symptoms after 5 and is
finished after 10 days. Any longer and the leaves are so encrusted with
deposits from the water that their appearance does not reflect blight
susceptibility. He has found a different reaction on leaves, flowers and
stems, similar to our findings.

Germain is going to start screening his selections for response to cold in
several new (and very nice) temperature controlled rooms. He is being very
cautious about releasing varieties, for fear that one bad year could wipe them
out and change the attitude of French growers towards walnuts. He is hoping
that French growers with his new varieties will be able to provide the EC with
all the walnuts they need. His only problem he says is the US selling cheaper
nuts, but he thinks we dry our nuts too much and there will be a taste
preference for the soggier French walnuts (why do we dry ours more?).

**Rootstocks.** English walnut was the common rootstock in France in the past.
For awhile *J. nigra* and the hybrid between *J. nigra* and *J. regia* was used,
but blackline was too widespread and Germain says he has had grafted trees
only 10 years old die from the disease. So the industry is back to *J. regia*.
They generally choose seed from Lozeronne because of its uniformity and high
percent germination. (We tried it and that is true but it is also of very
poor vigor.)

Germain has a program similar to ours for selecting tolerant clonal rootstock
from backcrosses. He is using *J. nigra* and *J. sieboldiana* instead of *J.
hindsii*, and seeks mostly vigor and blackline tolerance. (We hope to have
some resistance to Phytophthora and *rootability* as well.) His problem of
clonal propagation of rootstocks is the same as ours but the French have had a
good experience with micropropagation for other crops and are quite confident
in that method. It seems that if anyone will succeed with micropropagation it
should be the French.

**Propagation.** Standard propagation of varieties on seedling rootstock in
France takes more time than here. Bonilla's method might be adaptable to
France and if so will have a major impact on the cost of their trees and
consequently on their adaption of close plantings with their new varieties.

Clonal rootstock propagation and own rooted varieties are their current
concern. Germain believes that own rooted varieties will be much less
expensive so this year he began to put his advanced selections into culture.
The cultures that I saw were in sad shape with much contamination. Since Jay-
Allemand is such an expert in tissue culture I thought that Germain would have
better cooperation and success but he says Jay-Allemand is involved in
scientific research on tissue culture but not in the day to day application
(sounds familiar). For initiation into culture Germain first etiolates the
shoots in black plastic bags (for selections) or under big black plastic tubs
in the case of rootstocks. Success appears limited. But if he can get the material into culture he has a neat cooperative agreement to get it to the nursery.

Agri Obtentions of Dijon is a mostly self supporting INRA company that multiplies shoots for micropropagation. They sell the shoots for about $1.00 each to cooperating nurseries. This is working well for own-rooted chestnuts which are now commercially available.

The nursery we visited "Pepinieres Jean Coulie" is in the process of commercially propagating the chestnuts. Jean Coulie obtains boxes of shoots from Agri Obtentions and sticks the shoots, unrooted, into small containers (maybe 4X4X15cm) in 1/3 sphagnum + 2/3 pine bark, first dipping them for 15 seconds in 1000ppm IBA. They are immediately placed in a fog greenhouse for 6 weeks. The fog system was made by Mee industries of California and this nurseryman thinks it great. When we were there this fog "tunnel" was incredibly hot. It was 31C outside and felt 10C higher inside. Coulie says normally the temperatures range from 20C to 35C. After the 6 weeks the shoots have rooted and they are placed in the standard greenhouse under cloth. After 5 or 6 days the cloth is removed and the plants are maintained in the greenhouse until time for planting, usually the following year. The major problem is that the trees that overwinter in the greenhouse appear to go into dormancy normally but a good percentage of them leaf out and then die in the spring. Coulie is certain that the problem is not with water or applied nutrients; he thinks that in fact there is a problem of nutrient storage in the roots. Except for this problem he obtains about 70% success (out of more than 25,000 plants).

Coulie has worked with walnuts some. A few years ago he thought he could propagate walnuts by cuttings. He found that rooted cuttings have a poor root system, i.e. only a few roots from the cutting, often unbalanced. Micropropagated walnut shoots are more difficult to root than chestnuts, but Coulie hasn't put the same effort into it. He has found the same overwintering problem with the walnuts but believes that micropropagated shoots is the only way to go. He and Germain plan to cooperate on walnuts through Agri Obtentions.

One other method of propagation was of interest to me: mounding. I assume this is the standard method we can read up on. In this method the rootstock to be propagated is cut back almost to the ground and many shoots bud out from it. Wire is tied around the base of the shoots in May, leaves are removed partway up the stem, and dirt is mounded over so just the leafy part of the stem is uncovered. Several unwired branches have to be left to support the root system. Success is about 50% with 10 wired shoots per clump.

Training systems: We got to training systems late on a hot day. The slides I took explain Germain's experience with training systems better than I can. What was most interesting is that he is not only experimenting with different systems for hedgerow but also for evaluation in the breeding program. One study for hedgerows (Lara or Chandler) included a comparison of the US method with the French goblet and another French system that was notable for its minimal pruning. The two French systems resulted in earlier and higher yields. But then there was some problem with a frost that nipped the buds more on the US system. Another method I saw was in a younger trial so the
results are not in yet. This method is based on the training system used for apple. It looks like a V with the two main branches staked down. Yield is expected from vigorous shoots growing up from inside the V. Pruning is limited to cutting off the vigorous shoots once they lose vigor (2-4 yrs). Germain is very excited about this method.

For us, the way he trains trees for evaluation in the seedling and clonal selection blocks might be more interesting. His goals are to keep shoots easily in view and to be able to evaluate lateral bud fruitfulness accurately. He uses an axis system allowing long horizontal branches to push. For grafted trees it works like this: Yr 1) graft in nursery, July dormant bud; Yr 2) bud grows 3m?, dig; Yr 3) transplant, cut at 20 cm, new shoot will grow 2-3 m; Yr 4) encourage strong lateral shoots; Yr 5) evaluate lateral bud fruitfulness off these shoots.

Miscellaneous notes on varieties and crosses:

Ronde de Montignac. precocious and abundant pollen, long period of shedding, very vigorous

Meylannaise. another pollenizer, not precocious

Eureka. very susceptible to anthracnose

Serr. not susceptible to blight or anthracnose, self pruning, no PFA

Chico. leaves fall very late, very susceptible to fall frosts, not self pruning

Vina. not vigorous

Tehama. Germain likes it, self pruning

Cisco. bad twig blight, low yield

Chandler. great quality, moderate yields, self pruning

Lara. similar to 61-25, evidence of in breeding when crossed with Chandler, not self pruning

Moyer. weak but good pollenizer for Franquette

Grosvert-Verdot. good for the kernel market, i.e. nice and small for more kernels to the kg. light, plump and easy to remove. Not more than 11 g., makes a nice cross with Chandler

Lozeronne. rootstock, uniform and high % germination

Corne. rustic variety, vigorous in calcareous soil, hard shell, sounds like Poe

Parisienne. rustic variety, very vigorous in calcareous soil, big
nuts

Soleze.  Solignat found it. Germain released it to replace Corne. More vigorous and productive than Franquette, quality not as good, thin kernel

94-11.  good pollenizer for Franquette. will be released in 1996

94-12.  better yield and quality than 94-11, also will be released

Sieboldiana x regia.  easy to root

67-11 (Tulare).  great form