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Blight-Resistant Chestnut Selections of Switzerland: A Valuable Germ Plasm Resource

Chestnut blight, a lethal canker disease caused by the fungus *Cryphonectria (Endothia) parasitica* (Murrill) Barr P.J. & H.W. Anderson, has left an unsurpassed legacy of destruction in the forests of eastern North America. The fungus was introduced in the United States in the late 1800s, most likely on chestnut nursery stock from Japan, where the disease is endemic. The American chestnut, *Castanea dentata* (Marsh.) Borkh., proved to be so susceptible to this introduced disease that within 50 years one of the most economically and ecologically important tree species of the Appalachian region was virtually destroyed. American chestnut trees still exist as occasional stump sprouts within the native range and as isolated trees planted outside the range. These survivors offer the hope and possibility that the American chestnut may be resurrected through breeding for resistance or by exploiting the phenomenon of hypovirulence, or both. Hypovirulence is a debilitation of *C. parasitica* caused by the presence of a transmissible double-stranded RNA virus (1).

While *C. parasitica* was devastating the Appalachian forests, the fungus was inadvertently exported to Europe, where it infected another susceptible host, the European chestnut (*C. sativa* Mill.). When discovered in Italy in 1938, the disease caused a great deal of alarm because of the reported devastation in the United States. In southern Europe, the chestnut had been cultivated on the mountain slopes for centuries as a provider of food, fodder, and timber. The chestnut often occurred as pure stands on steep slopes. Destruction of the chestnut forest would be an economic disaster for those who depend on chestnuts, and the denuded mountain slopes would be subject to erosion, mud slides, and avalanches.

Chestnut forests were of special concern in Switzerland, which has had a long-standing governmental policy of forest protection. In the southern canton of Ticino, where chestnut forests are common, foresters watched the spread of the disease epidemic as it crossed the border in 1947. Confronted with what was perceived as a dire emergency, the Swiss Federal Institute of Forestry Research began an extensive 30-year program to select blight-resistant chestnut trees (Fig. 1).

At the outset, the goal of the research was to save the chestnut from devastation by *C. parasitica*. Because the situation was urgent, the strategy was to select as many resistant trees as possible, as quickly as possible. During the three decades of selection, however, the situation changed in two respects. First, the blight epidemic in Europe was less severe than its counterpart in North America. Chestnut trees suffered much damage and many died, but the chestnut forests were not completely destroyed, as had been feared. The less severe European epidemic may have resulted from climatic differences, higher blight resistance of *C. sativa* compared with *C. dentata*, debilitation of *C. parasitica* by transmissible hypovirulence (3), or a combination of these factors. Because European chestnut stands were not destroyed, there was no need to replace large areas of chestnut forest with resistant trees. Second, as the research progressed, the economic importance of the chestnut declined along with interest in chestnut plantings and chestnut improvement.

In recent years, worldwide interest in chestnuts has been increasing, along with renewed interest in the program as a source of information and germ plasm. Although much of the information and genetic material that is now desired cannot be provided, this program comprises the most extensive chestnut blight resistance selection thus far carried out. Previous publications (2,5,7) have described the detailed phytopathological studies and artificial inoculation methods developed during this research program. In this article we summarize the origin, characteristics, and current status of the selected chestnut population.

The Genetic Base and Initial Selection

The early years of the program were devoted primarily to phytopathological studies and development of a reliable screening technique that utilized artificial inoculation (Fig. 1). After these preparatory studies, large-scale screening of chestnut seedlings was begun. To provide a chestnut population for screening, seeds were collected from *C. sativa* populations in Canton Ticino (Fig. 2) and a few sources in Canton Valais, Canton Zug, and south-central France. In addition, *C. crenata* Siebold & Zucc. seeds were obtained from old trees (more than 40 years old) growing in the Ceneri region of Canton Ticino and from young nursery trees in Canton Zurich. Seeds of *C. mollissima* Blume and hybrids were obtained from the Connecticut Agricultural Experiment Station in the United States. During the selection phase, many candidate trees and selections began to bear seeds. Seeds collected from these trees were planted and entered into the screening process as second-, third-, or fourth-generation candidates. Nearly all seeds in all four generations resulted from open pollination so that the pollen parents were unknown.

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At 4–5 years of age, each candidate tree was inoculated in May or June. A small bark disk was removed, the hole was filled with cultured, virulent mycelium of *C. parasitica*, and the wound was taped (7). Between 1962 and 1972, more than 120,000 seedlings were inoculated by this technique in experimental nurseries in Canton Ticino. Approximately 14,000 trees survived the subsequent infection and were planted in permanent field plots (inventory stands).

Because all offspring of many *C. sativa* mother trees died after inoculation, the inventory stands represent only part of the populations originally sampled. More than 300 mother trees from the populations originally sampled (G_0) had one or more offspring that survived the inoculation test. These survivors were the first-generation (G_1) selections. Assuming that pollen came from trees other than the mother trees, the total base population (G_0) was probably between 600 and 1,000 trees. No effort was made to equalize the number of progeny from each mother tree. From some trees, as many seeds as possible were collected each year. From others, seeds were collected only once. Therefore, the G_1 population was genetically unbalanced with respect to the base population because of different numbers of progeny (family sizes) and selection for blight resistance.

In the advanced generations (G_2, G_3, G_4), there was an additional selection for precocity, because seeds were collected only from trees that began to bear at less than 10 years of age. During four generations, approximately 1,700 mother trees contributed one or more selections. Most of the families were small; 500 mother trees had only one selected offspring and 750 trees bore two to 10 selections. However, there were 34 large families of 50 or more selections.

The selection for blight resistance and precocity seemed to favor germ plasm of *C. crenata*, because overwhelmingly large numbers of advanced-generation selections were derived from *C. crenata* (Table 1). On the other hand, surrounding stands of large *C. sativa* trees probably contributed much pollen, especially in the first generation. Although most of the selections are hybrids between *C. sativa* and *C. crenata*, or are derived from such hybrids, some first-generation selections are pure *C. sativa*. The advanced-generation seeds were collected from very densely planted and somewhat isolated nurseries, which probably resulted in considerable inbreeding.

The survival rate of inoculated seedling populations varied with seed source and environmental conditions in the nurseries (year-to-year variation). Unfortunately, data on the inheritance of blight resistance were not collected, but the available data do provide some useful information. There was no detectable geographic variation in blight resistance of *C. sativa* (2). Typically, the survival rate of inoculated *C. sativa* seedlings was in the range of 1–5%, whereas the survival rate of inoculated *C. crenata* and *C. mollissima* seedlings was about 10-fold greater. The chestnut populations derived from Asiatic mother trees were much more variable in survival rates, however, perhaps as a consequence of hybridization with *C. sativa* as well as inherent genetic variability in blight resistance.

Year-to-year variation in survival rate was considerable. For example, in 1961 and 1968, less than 5% of the seedlings from open-pollinated *C. crenata* and *C. mollissima* survived, while in one nursery in 1963, more than 60% survived (sample sizes were more than 1,000). Year-to-year fluctuations in survival rates of open-pollinated seedlings of *C. sativa* generally followed the same pattern but were not as great. Thus, inoculation tests were as much a measure of physiological predisposition as a measure of genetic resistance (2,5). One obvious consequence was that there were many more selections in the years when few trees died than in the years when many died. Thus, the selected population was not uniformly resistant.

Plantations of Selections Resistant to Blight

The 14,000 survivors of inoculation tests (selections) were planted in permanent field plots (inventory stands), either as vegetatively propagated clones or as single trees. Counting clonal replicates, more than 31,000 trees were planted in 19 inventory stands during a 9-year period (Table 2). Clones usually were propagated by layering (4); a few were grafted. Generally, each clone was planted in only one inventory stand, but a few were planted in two or rarely three stands. The number of replicates of each clone varied but generally was fewer than 10. In one inventory stand (Cademario), unscreened seedlings from selected mothers were planted between the selected trees.

In the first years of selection, when selections were comparatively few, a higher proportion were vegetatively propagated. Later, the large numbers of selections precluded clonal propagation, and they were preserved only as single trees. These differences in clonal replication may explain the fact that survival rates in the inventory stands have been highest for first-generation selections and lowest for third-generation selections (Table 1).

The main purpose of the inventory stands was to serve as clonal repositories for the large number of selections emerging from the program. Therefore, trees were not planted in an experimental design. The planting distance was generally 2 m, except for a few special nut-tree plantings at Gorduno and Gambarogno where spacing was wider. The planting sites were generally on moderate to steep slopes in areas where chestnuts are a large component of the natural forest. Each tree was numbered and mapped. Three and 10 years after planting, data collected from all trees included height, diameter, blight cankers and injury, mortality, and

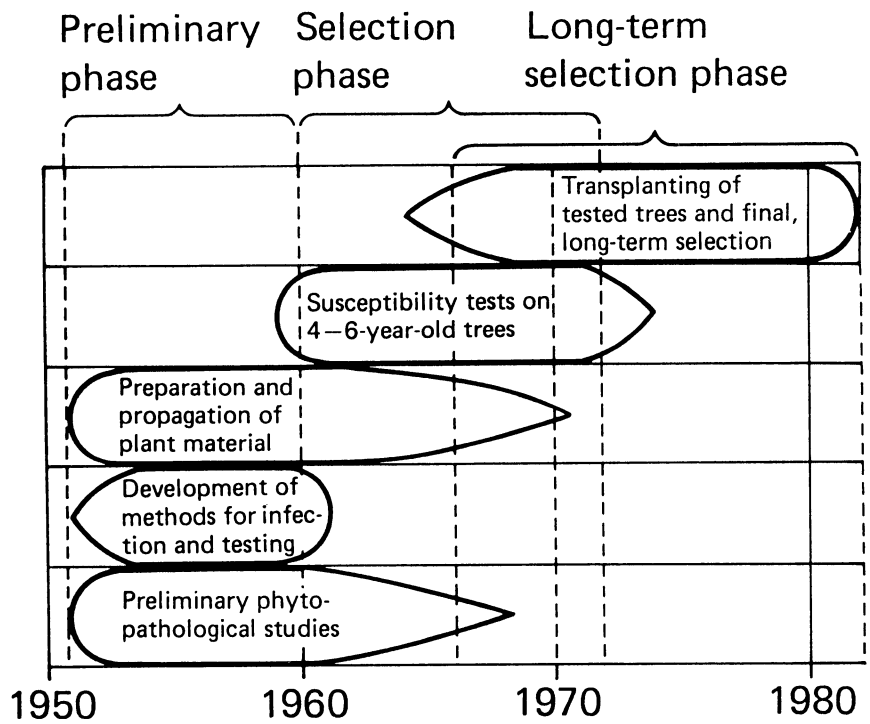

Fig. 1. Time schedule of chestnut blight resistance selection program conducted by the Swiss Federal Institute of Forestry Research.

cause of death. A computer record for each tree combined the tree's field data with its origin (mother tree and generation number).

Because of unequal family sizes, unequal replication of clones, and lack of experimental design, these voluminous data have limited usefulness. Evaluation of growth data was further complicated by heterogeneity of the planting sites. Differences in mean 10-year height and diameter at each of the inventory stands (Table 2) suggest a large degree of heterogeneity among stands, although genetic and weather factors cannot be excluded as contributors to differences observed. There was also obvious within-stand variability, apparently related to such things as topography, aspect, and soil depth. The close spacing has precluded a meaningful evaluation of fruiting characteristics, especially of smaller trees that have been shaded by their neighbors.

Subsequent Selection

Originally, the only deliberate selection criterion was blight resistance; other characteristics were not considered. Since planting, many of the inventory stand trees have died from various causes (Tables 1 and 2), resulting in a quasi-natural selection. After 3 years, 21% of the trees had died, approximately one-third from blight and most of the rest from unknown causes, probably environmental stresses related to transplant shock or poor site conditions. By the 10-year measurement, 12% of the living trees had been removed as part of silvicultural thinning, and an equal number had died of natural causes, including 3% killed by blight. The exact number of deaths attributable to *C. parasitica* was difficult to determine, especially at the 10-year measurement. Since the 10-year measurement, many trees, especially the smaller ones, have been crowded out because of close spacing.

During the first 10 years, there was generally a negative correlation between the mean growth rate of living trees and the proportion of trees that died from blight. In other words, sites that supported faster growth lost fewer trees to *C. parasitica*. Interestingly, the proportion of deaths from other causes was not correlated with growth rate.

Many of the trees that died were probably genetically less fit with respect to blight resistance, growth rate, growth form, or environmental adaptation. To the extent that the selection was genetic, the population has been genetically improved, at least with respect to desirable silvicultural characteristics. Because the survivors after 10 years represent a large portion of the original selected families, this selection has not resulted in a great reduction of genetic diversity. However, because of close spacing and silvicultural treatment of the stands, some good nut-producing clones may have been lost because of their nontimber growth form.

In 1986, a sample of clones was selected to provide a representative population of manageable size for future research and evaluation (6). The goal was to select 100–200 of the "best" clones from a genetically diverse background. Statistically significant growth differences among families and clones indicated that there were genetic differences amenable to selection. A computer-generated list of candidates, combined with direct observations in the field, provided the basis for selection. Direct field observations in the early spring proved to be the fastest and simplest way to make selections, since hundreds of trees could be observed from one vantage point and many factors and characteristics could be simultaneously considered. The trees were selected on the basis of relatively large size, freedom from blight cankers, and wide branch angles. A typical *C. sativa*-type growth habit (straight bole, wide branch angles, good apical dominance) was preferred, but other growth habits were considered acceptable if the trees showed evidence of heavy or large nut production. A total of 120 clones was selected, i.e., about 1% of the original selections.

At the time of the 1986 selection, most of the inventory stand trees were found to have blight cankers of varying severity. Also, many trees were very small or had very poor stem form. These trees were easily eliminated from consideration, but because the general condition, age, and site differences within the stands were all variable, the selection tended to be subjective. Therefore, the 120 selections were considered to be a representative sample of "good" clones, but not necessarily the "best" 120 clones. The 120 selections represented more than 80 different families and were equally

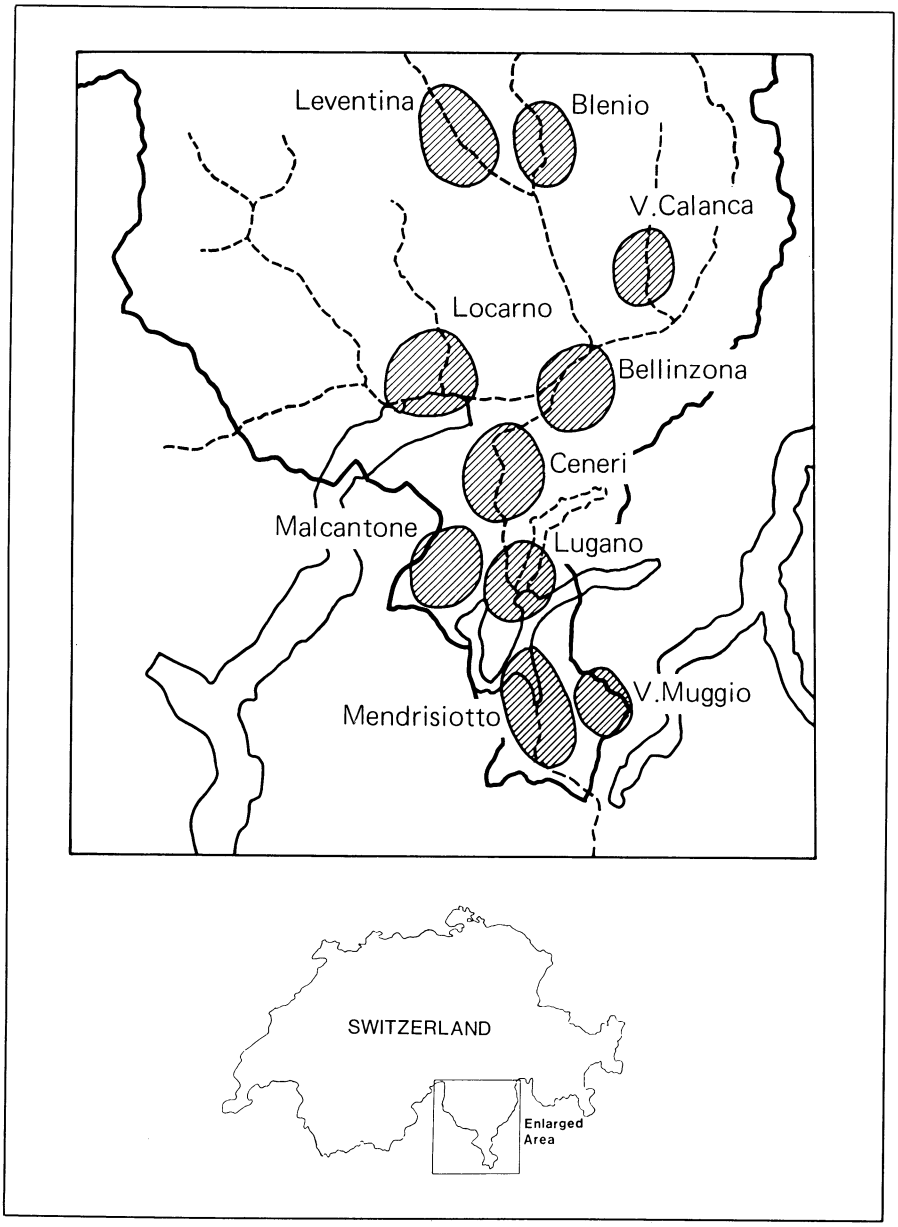

Fig. 2. Regions (shaded areas) in Canton Ticino where chestnut seeds were collected for subsequent blight resistance selection.

Table 1. Number of chestnut trees (*Castanea* spp.) that contributed offspring to be screened for resistance to *Cryphonectria parasitica* (G_0) and numbers of selected offspring per generation (G_1, G_2, G_3)[a]

Base population trees (G_0)			Number of selections per generation					
Species Source	Number		G_1		G_2		G_3	
Castanea sativa								
Ceneri	29	(24)[b]	306	(252)	73	(45)	324	(241)
Mendrisiotto	11	(11)	35	(31)	11	(9)	217	(183)
Valle Muggio	2	(2)	3	(3)	2	(2)	0	(0)
Lugano	17	(16)	108	(98)	1	(1)	9	(7)
Mulcantone	12	(5)	14	(5)	3	(2)	122	(88)
Locarno	11	(9)	103	(90)	41	(32)	0	(0)
Leventina	6	(6)	25	(24)	0	(0)	0	(0)
Bellinzona	11	(11)	328	(261)	57	(45)	69	(56)
Blenio	1	(1)	0	(0)	0	(0)	32	(27)
Valle Calanca	2	(2)	66	(47)	0	(0)	0	(0)
Monthey (Canton Valais)	1	(1)	10	(9)	3	(3)	5	(3)
Walchwil (Canton Zug)	1	(1)	1	(0)	31	(23)	52	(37)
France (Massif Central)	2	(2)	48	(45)	0	(0)	0	(0)
Unknown	125	(99)	151	(111)	8	(8)	88	(61)
C. crenata								
Ceneri	26	(26)	306	(203)	1,740	(1,361)	7,864	(5,762)[c]
Zurich	33	(31)	303	(269)	110	(89)	298	(198)
C. mollissima								
Connecticut, USA	11	(11)	186	(152)	437	(290)	115	(65)
Hybrids								
Ceneri	6	(6)	0	(0)	5	(3)	145	(103)
Bellinzona	1	(1)	3	(3)	0	(0)	0	(0)
Zurich	2	(2)	2	(2)	0	(0)	0	(0)
Connecticut, USA	12	(12)	58	(40)	214	(150)	652	(522)
Total	322	(279)	2,056	(1,645)	2,736	(2,063)	9,992	(7,353)

[a] G_0 mother trees were not screened for resistance and were not planted in inventory stands; selections were planted in inventory stands. G_1 selections were open-pollinated progeny of G_0 mother trees that survived inoculation with virulent strains of *C. parasitica*. G_2 and G_3 selections were open-pollinated progeny of G_1 and G_2 mother trees, respectively, that survived inoculation with virulent strains of *C. parasitica*.

[b] Numbers in parentheses indicate the number of survivors after 10 years in inventory stands or, for G_0 mother trees, the number having surviving offspring in the inventory stands after 10 years.

[c] This group included 82 G_4 selections with 61 surviving after 10 years in the inventory stands.

Table 2. Inventory stands, establishment years, number of trees (including clonal replicates), and mean 10-year height and diameter at breast height (dbh)

			Number of trees		After 10 years	
Location	Stand number	Year planted	Initially planted	After 10 years	dbh (cm)	Height (dm)
Copera	49	1963[a]	788	498	3.16	30.8
Quartino	50	1964	775	464	5.59	44.5
Quartino	54	1967	1,534	1,093	3.32	36.4
Sagno	55	1964	1,761	1,369	4.58	40.3
Cabbio	56	1964	986	851	4.31	38.0
Cabbio	57	1964	167	137	4.05	34.4
Gambarogno	60	1965	100	84	4.19	37.9
Gambarogno	61	1965	77	64	5.03	43.6
Quartino	63	1966	189	165	4.98	57.7
Gambarogno	64	1966	600	226	3.36	44.0
Aurigeno	65	1967	1,292	712	4.58	45.8
Aurigeno	66	1967	418	335	5.30	65.8
Osogna	67	1967[a]	201	106	7.05	57.6
Cademario	68	1968	6,376	3,318	5.56	62.2
Aranno	69	1968[a]	1,526	847	4.80	43.2
Gorduno	70	1969[a]	569	358	5.01	42.4
Osogna	71	1970	7,819	3,152	6.47	57.0
Magadino	72	1971	3,646	1,953	5.17	59.1
Golino	73	1971	2,759	1,629	4.62	46.2
Total			31,583	17,361		

[a] Replacement trees were planted in 1970.

divided among each of three generations. They show much variation in morphological characteristics. Thus, even this small sample seems to be genetically diverse.

Discussion

The high frequency of blight-cankered trees in the inventory stands was surprising, considering the severity of the initial selection, which usually killed more than half of the offspring from *C. crenata* and *C. mollissima*, the species most resistant to chestnut blight. Possible explanations are that: 1) during the years when many seedlings survived inoculation (as in 1963), a large number of trees with low resistance may have survived; 2) some of the selected seedlings may have been escapes, the result of poor inoculations; and 3) because of their hybrid or exotic background, poor environmental adaptation might have predisposed them to subsequent attack by *C. parasitica*. Environmental stresses increase predisposition to attack by *C. parasitica* (9). The inventory stands frequently have been exposed to drought (and occasionally fire), limb breakage from heavy snow loads, and bark damage from hail and woodpeckers. Also, the high plantation density, silvicultural pruning and thinning (leaving dead wood in the plantation), and presence of susceptible trees provide a large reservoir of inoculum and opportunity for rapid spread of *C. parasitica*. Finally, trees with moderate resistance often develop cankers that are restricted to the outer bark. Thus, the presence of superficial or swollen cankers may be an expression of resistance (or indicate the presence of hypovirulence).

Despite the high incidence of chestnut blight, several hundred clones had no blight cankers or only sublethal cankers. Considering their original selection, plus 15–25 years of exposure to *C. parasitica* in the field, most of these trees probably have some degree of resistance. Within this population were many individuals with good timber and/or nut-producing characteristics. There was no observable correlation between growth form and blight incidence, as was reported by Jaynes (8). Some trees had a typical *C. sativa*-type growth habit and growth rate, but hybrid (*C. sativa* and *C. crenata*) twig characteristics. Thus, it seems that timber-type trees can be recovered from *C. sativa*/*C. crenata* hybrids within one or two generations, if segregating populations are large enough.

The 30 years of experience in this program and the results of other chestnut research (9) indicate that there are only subtle differences between blight-resistant and blight-susceptible chestnut trees. There appears to be a continuous gradient from very susceptible to very resistant, but no complete immunity. Environmental conditions and physio-

logical status of the tree greatly influence canker severity. Consequently, evaluation of and selection for blight resistance are very difficult.

This program has produced a large number of seemingly blight-resistant chestnut clones that also have good environmental adaptation, rapid growth rates, and various growth forms, including good timber forms. These selections represent the broad genetic diversity of a large base population, including three species. However, confirmation of blight resistance and evaluation of other characteristics are necessary before the true genetic value of the population or specific clones can be assessed. This population is a potentially valuable germ plasm source for Switzerland and also for other parts of the world.

Acknowledgments

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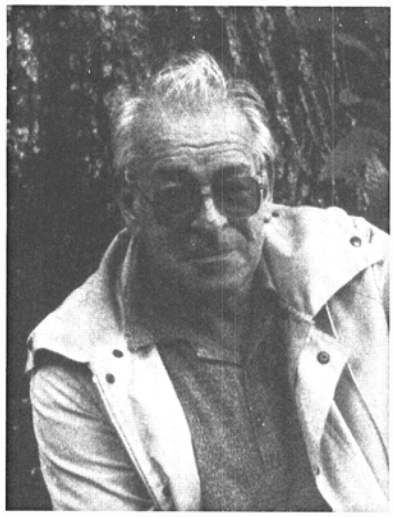

Giovanni Bazzigher

After receiving a bachelor's degree in agriculture from the Swiss Federal Institute of Technology, Dr. Bazzigher earned his Ph.D. degree in 1953 with the late E. Gäumann with a dissertation on the biology of *Cryphonectria (Endothia) parasitica*. That same year he joined the Swiss Federal Institute of Forestry Research (now the Swiss Federal Institute for Forest, Snow, and Landscape Research), where he held the position of forest pathologist, with both extension and research responsibilities, until his retirement in 1987. After the introduction of chestnut blight in southern Switzerland, he investigated the biology of the disease, selected trees for resistance, and studied the phenomenon of hypovirulence. He also investigated tree diseases of the subalpine region (Scleroderris canker, snow mold) and butt rots of conifers.

Gregory A. Miller

Dr. Miller completed a combined bachelor's and master's degree, specializing in plant breeding and genetics, in the Horticulture Department of The Ohio State University in 1978. He received a doctorate in 1983 in the Forestry Department of Iowa State University, specializing in forest genetics and tree breeding. Since then he has worked full time on chestnut production and research. In 1986 he spent 6 months as a visiting scientist working with Dr. Bazzigher in the Swiss Federal Institute of Forestry Research. Currently, Dr. Miller is the owner of Empire Chestnut Company, a family farm/business located in east central Ohio. The primary activity of the business is research related to commercial chestnut production, afforded largely by Phase I and Phase II grants from the USDA Small Business Innovation Research Program. The goal of the business is to demonstrate commercial feasibility of chestnut production and to address technical problems that currently limit chestnut production in the United States.