Chapter 10

Resistance breeding against tree pathogens

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1. Introduction

Forest health and its associated economic and ecologic benefits, including ecosystem services, are under constant threat by invasive pathogens and pests, many of them non-native (Aukema et al., 2011; Lovett et al., 2016; Liebhold et al., 2017). If a destructive pathogen or pest becomes well-established, there may be little management can do to protect and save the affected species, including the large, old-growth trees, except to develop a resistance program, and use the seed for restoration or reforestation. Fortunately, the extant genetic variation within tree species offers hope that with a well-designed and well-focused resistance breeding program, resistant trees can be identified, selected, and bred to produce populations with resistance levels necessary to save and restore the species.

Resistance breeding is an action-oriented effort that is solution-minded—countering the impact of a major disturbance caused by a disease or pest. Recent successes in forest tree resistance breeding (Sniezko and Koch, 2017; Sniezko et al., 2014; Pike et al., 2021) have been joined with a growing recognition that breeding can and should play a more prominent role in ensuring healthy forests to meet society's needs (Bonello et al., 2020; Buggs, 2020; Federman and Zankowski, 2020; Koch et al., 2021; Naidoo et al., 2019; Pike et al., 2021; Showalter et al., 2018; Sniezko and Koch, 2017; Telford et al., 2015; Woodcock et al., 2018; Yanchuk and Allard, 2009; Nelson and Koch, 2017).

Tree improvement, including resistance breeding against tree pathogens, has been underway in several species for more than 50 years (Bingham et al., 1972; Gerhold et al., 1966; Heybroek et al., 1982; Nelson et al., 2020; Sniezko et al., 2012c; White et al., 2007; Zobel and Talbert, 1984). Resistance tree breeding programs have included (a) native tree species and native pathogens or pests, (b) native tree species and non-native pathogen or pests, and (c) non-native trees used in managed plantations. In most cases, resistance programs have been undertaken for species of high economic value, but more recently, programs for tree species of high ecological value have also been included (Koch et al., 2021; Sniezko and Koch, 2017; Pike et al., 2021).

The success of a resistance breeding program depends on several factors, including allied research; access to tree improvement infrastructure; engaged technical specialists, including pathologists and entomologists; and continuity of administrative and public support (Fig. 10.1). The technical side of the success can be considered in two stages: (1) exploration to assess whether genetic variation in resistance exists in the species and (2) development, using tree improvement practices, such as selective breeding, to apply the knowledge gained in the first stage to develop resistant planting stock for use in restoration. To be successful, a resistance program also needs a sense of urgency and keen focus on the end product, specifically, the delivery of genetically diverse, resistant seedlings for planting in a relevant timeframe.

Trees are perennial and long-lived (many decades to centuries) and must thrive on the landscape in the face of various biotic and abiotic stressors. The intent of a resistance breeding program should be to develop genetically resistant populations of trees that are well adapted to the local environments and include sufficient genetic diversity to allow them to survive and evolve in the face of various future abiotic and biotic challenges. Fortunately, having answers to the basic research questions relating to the underlying mechanisms of the resistance is not immediately necessary for resistance breeding to progress. The successful programs to date have generally focused on developing genetically resistant populations first and only later determining details of the mechanisms and inheritance of the obtained resistance.

Although there are similarities between agricultural crop and forest tree resistance breeding, there are also important differences. In agricultural crops, one or a few cultivars planted in a field can generally meet the producers' needs, including product uniformity. Many crops are also annual species and can be planted each year, with different or even new cultivars substituted. In contrast, planted forest trees are intended to be on the landscape for decades to a century or more, and in the case of naturally regenerated forests or wildland areas, they will be the parents of the succeeding generation. So, although

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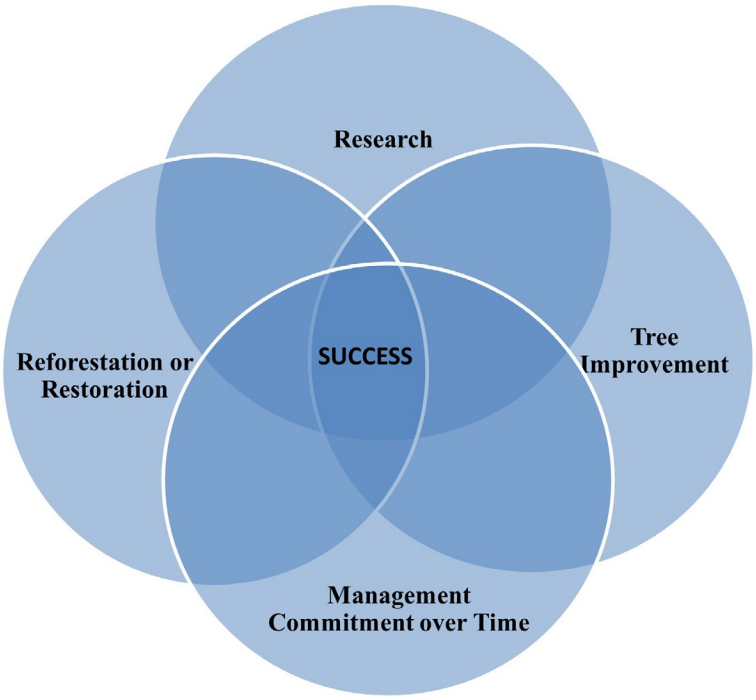

FIG. 10.1 Components necessary for developing successful resistance breeding programs. *Adapted from Sniezko, R.A., Koch, J. 2017. Breeding trees resistant to insects and diseases—putting theory into application. Biol. Invasions 19, 3377–3400. https://doi.org/10.1007/s10530-017-1482-5.*

durability of resistance (resistance is persistent over time) is important in crop plants, it is essential in forest trees since in many cases, planted trees will also be the progenitors of the future forests through natural regeneration. Stability of resistance (resistance is consistent over environments) especially under a changing climate is also essential. Unlike agricultural crops, cultivars bred for uniformity are not generally desired and are not used for most forest trees. The allowed and desired heterogeneity in resistant tree populations can help facilitate the development of durability and stability through using both major gene resistance (MGR) and quantitative resistance (QR). It is also important for naturally regenerated forest tree populations to pass their superiority (e.g., increased resistance) to the next generation or to be true breeding as well as heterogenous.

The nature of each pathosystem, and the level of resistance available in the first generation after selection, will vary widely by tree species and disease. In many cases, few seedlings will exhibit resistance (e.g., expected to survive to reproductive age under disease pressure) following a natural infestation or artificial screening (signs of resistance may be very subtle), but the few that do need to be identified and propagated to serve as next-generation parents. In general, the population used for planting will come from seed orchards consisting of the selected parents where QR often provides the major component of the observed resistance. In these cases, seeds/seedlings produced by the orchards will have varying levels of resistance. Seedling survival from the orchard will be higher than wild type or susceptible controls, but to be clear, it will vary by species and the level of disease pressure. Where the resistance level is too low, selective breeding (crossing selected trees with each other) can be used to increase the level of resistance available in future seed orchards.

The successes to date in forest tree resistance breeding have generally been obtained using classical tree improvement, that is, without the aid of modern biotechnologies such as genetic engineering or even marker-assisted selection (MAS). Advances in biotechnology may lead to acceleration of the development of resistance, including the use of genetic engineering to add resistance gene(s) that are not present in the tree species (e.g., Powell et al., 2019). However, there are numerous social, ethical, and technological considerations when choosing to utilize genetic engineering or other biotechnological tools (National Academies of Sciences, Engineering, and Medicine, 2019). On the technological side, is the need for congruent integration of biotechnology with active tree improvement programs (Nelson, 2021), but unfortunately, the number of such programs has diminished greatly over the last several decades (Wheeler et al., 2015). Forest genetics and tree improvement programs will need to be strengthened or rebuilt if we are to reap the benefits that only they can convey in the presence of diseases, pests, and a changing climate (Nelson and Koch, 2017; Pike et al., 2021). The time to develop resistant populations can vary from perhaps a decade or less to several decades, depending on the resources made available,

pathosystem specifics, and the level of resistance ultimately required. This timeframe is reasonable, being not much different than cultivar development in many agricultural crops or even the development of shorter-term pest management tools in forestry. As with other aspects of resistance breeding programs, the timeline to deliver acceptably resistant planting stock should be developed with stakeholder input and subsequent feedback as the program progresses.

As we discuss in this paper, applied disease resistance breeding programs in forest trees have been underway in the United States for more than 50 years. The extensive experience and knowledge gained in a few of these programs is highlighted to encourage the initiation and development of new resistance breeding programs and to provide context and insights to ensure the success of current and future programs. Our focus is on disease resistance, but most of the concepts would also apply to developing resistance in forest trees to other biotic as well as abiotic threats.

2. Components and stages of resistance breeding

2.1 Initiating a program

To be successful, a resistance breeding program needs high societal interest in the problem to be solved, strong management support, and a well-organized effort focused on delivering a solution (genetically resistant trees, regionally adapted, and appropriately diverse) in a relevant timeframe. An applied program generally differs greatly from a research project in objectives as well as in the magnitude and focus. Since resistance can be rare, hundreds to thousands of candidate trees (or their progenies) might be needed to be screened to provide enough resistant trees for the diverse genetic base required for reforestation.

The basic facilities to run a program might include (a) greenhouses or other related growing space, (b) seed handling and cold storage capacity, (c) inoculation infrastructure, (d) field sites for testing, (e) database capability for collecting, maintaining, and analyzing data, and (f) areas for seed orchard development. Personnel needs will include a tree breeder, data manager, technicians, and administrative support personnel, as well as access to pathology (and entomology) expertise and support. The tree breeder will provide planning and guidance in the overall program; the pathologist in collaboration with the breeder will provide protocols for seedling inoculation and evaluation; the technicians will provide much of the core effort to advance the program. As with any tree improvement effort, some basic knowledge of the biology of the tree species will be essential, including information on the distribution of genetic variation that may allow initial seed and breeding zones to be established (Campbell and Sugano, 1989). Although a single organization may be able to carry out a program, the incorporation of other partners or cooperators with an interest in the species can provide much needed assistance and increase the potential for success (Nelson and Koch, 2017; Pike et al., 2021). As examples, the USDA Forest Service (USFS) resistance programs in the Pacific Northwest region have benefited greatly from such cooperative support, and it is also a key to the university-industry tree improvement cooperatives in the southern United States, and the Great Lakes Basin Forest Health Collaborative provides another example (https://holdenfg.org/great-lakes-basin-forest-health-collaborative/).

2.2 Implementation phases

A general framework for resistance breeding programs is provided in Fig. 10.2. It is helpful to view a resistance program as a two-stage process: an exploratory phase (Phase 1)—recognizing and evaluating resistance, and a developmental phase (Phase 2)—selecting resistant genotypes to serve as parents and producing resistant planting stock. A resistance program may involve several cycles of breeding, and much of Phase 1 occurs in the early stage of the first cycle (Fig. 10.2).

In Phase 1, one would like to (a) locate candidate resistant trees, preferably in each geographic area heavily impacted by the pathogen or pest, (b) develop a short-term assay(s) or screen to reliably assess genetic variation in resistance, (c) determine the types/levels of resistance (e.g., qualitative or quantitative, or both), (d) determine the geographic distribution of resistance, and (e) establish initial field trials to validate the screening assay as well as to monitor for durability and stability of resistance. Major objectives of this phase include determining whether genetic resistance to the disease exists and at what level in the various populations, beginning to evaluate the durability and stability of resistance, updating stakeholders with summaries of results and details as needed, and deciding whether and how to proceed to Phase 2.

Phase 2 capitalizes on the information gained during Phase 1, as well as the knowledge of the tree species' biology, understanding of tree improvement principles and practices to (a) scale up and operationalize the screening protocol and efforts, (b) select resistant candidates, or some of their progeny (forward selections), using the screening protocol to evaluate hundreds to thousands of trees, (c) establish seed orchards or other methods to deliver resistant planting stock, and (d) establish additional field trials for further validating and delineating resistance.

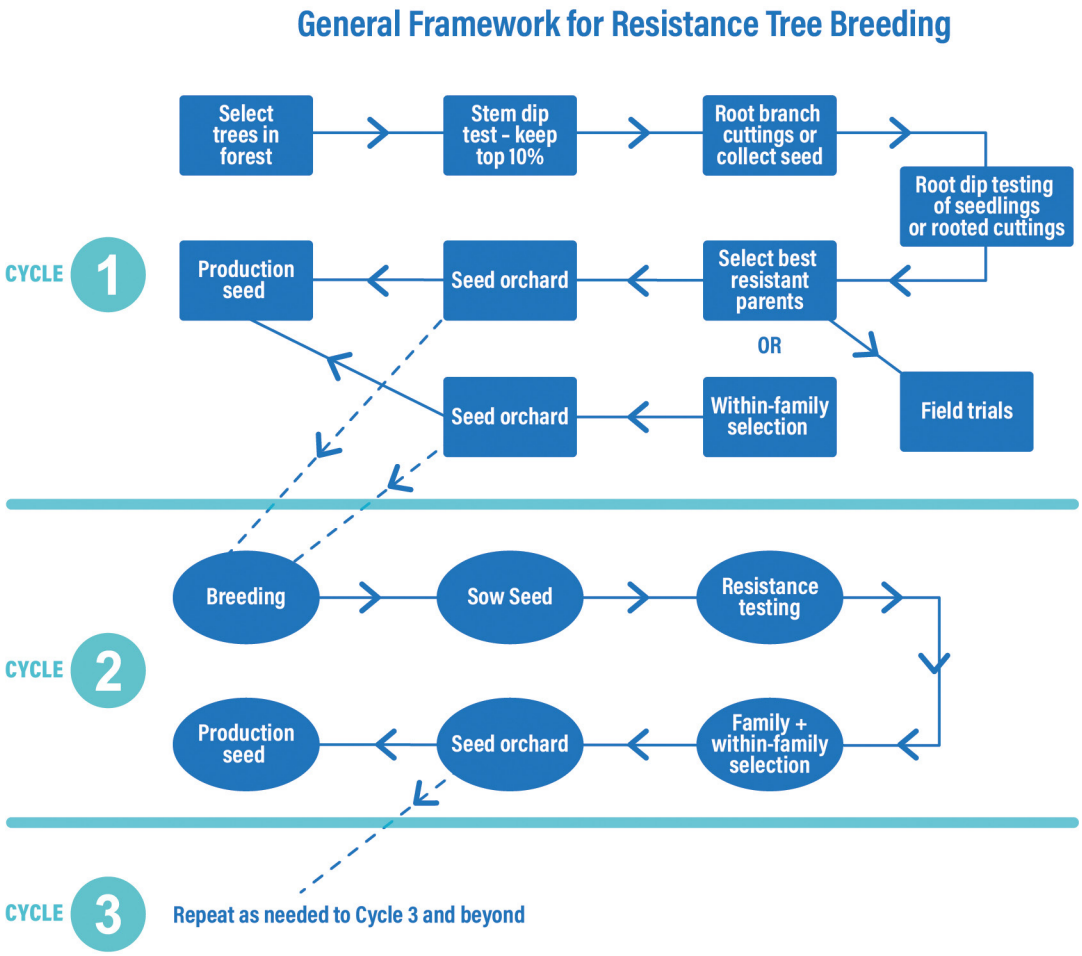

FIG. 10.2 General framework of a resistance tree breeding program showing successive cycles of selection, testing, and breeding for resistance. This framework is currently being used to develop resistance to *Phytophthora lateralis* in Port-Orford-cedar (POC; *Chamaecyparis lawsoniana*) (see section on case studies and Fig. 10.4). In Cycle 1, the initial tasks include making field selections, testing for the presence of resistance using greenhouse trials, noting the presence of any geographic variation in resistance, initiating the first field trials to confirm the efficacy of greenhouse trials and to begin monitoring durability of resistance, and assuming resistance is detected, starting the first seed orchards. Subsequent cycles use the base population developed in Cycle 1 or earlier (including current seed orchard trees) to begin breeding (dashed lines in figure moving program from one cycle to the next) to increase resistance levels and develop improved seed orchards to deliver seed with higher levels of resistance.

In summary, the basic steps for resistance breeding are the same for each pathosystem: identifying and quantifying genetic variation and selecting and increasing resistant trees in a way that are useful to the overall objective (solving the forest health problem). However, each tree species has unique biology, and this may influence the frequency, level, and distribution of genetic resistance, as well as the timeline needed to begin releasing and increasing resistant planting stock. A forest tree resistance program is a solution-oriented step to a serious natural resource problem. For it to be successful, a core group of stakeholders must realize the problem, acknowledge that resistance breeding offers the best avenue for maintaining the species of concern, and express a willingness to invest in a solution that could take one or more decades. Given the decade(s) timeframe, continuity of a resistance program is also essential to success.

2.2.1 *Phase 1—Exploratory: Early screening and field testing*

The development of resistant populations will generally require evaluating hundreds to thousands of candidate parent trees or their progenies under high, uniform disease pressure. In the optimum case, the candidate trees are selected from areas in which the disease has already taken a heavy toll and thus provided some preselection (Johnson and Sniezko, 2021); in other cases, the selections will be more random, and many more trees may have to be tested. Developing an effective screening protocol to evaluate seedlings accurately is a key step to get right: a weak screen may select too many trees (resulting in selections with little to no resistance), at the other extreme, too strong of a screen may kill too many seedlings (masking subtle

signs of resistance), or a screening assay may relate poorly to resistance that occurs under field conditions. As noted below in the Port-Orford-cedar (POC; *Chamaecyparis lawsoniana*) example, early work on screening did not end up reflecting the real story on resistance (to *Phytophthora lateralis*). Although it might be desirable to have an early and fast screen, this should not be at the expense of uncovering the full range of genetic variation in resistance that may exist. Once a screening system is optimized for its objective of identifying genetic variation in resistance, then methods to potentially accelerate the screening protocol (or phenotyping individual trees) can be explored.

Field trials are essential to validate the short-term screening and ensure that resistance holds up under natural conditions of prevailing disease pressure, as well as ensuing that the resistance is durable and stable. Once established, field trials can take several years to a decade or more to express high levels of infection (on susceptible controls), but this can be facilitated by selecting some sites of known high disease pressure, or purposely enhancing the disease hazard at the test sites. Including both known susceptible and resistant families (checklots) as controls is valuable for making comparisons and ensuring the efficacy of each trial. Resistant checklots should include families with a range of resistance types, ideally representatives with MGR and QR if both types are known to exist. The highly susceptible checklots will provide a monitor (positive control) on the disease pressure for the tests and provide a benchmark for comparison with resistant families. The identified resistant parents can then be selected for use in seed orchards or as candidates for next-generation breeding. Demonstration plots that showcase the level and effectiveness of resistance can be key to building and maintaining support for the program. Another function of field trials or demonstration plots will be to examine whether there are any negative correlations between resistance and other desirable traits. Orchard seed from several resistance programs has been used in managed forests for many decades, and in general, the widespread adoption by forest managers strongly suggests that there does not appear to be any noticeable negative impacts from selecting for resistance. However, monitoring the performance of resistant stock early in the program and rogueing any individual parents with notable poor growth or other undesirable traits will minimize the possibility.

For at least some non-native pathogens, greater than 95% of trees and seedlings may be highly susceptible, and land managers will be reluctant to use the species in plantation forestry or restoration without improvement in the level of resistance. In the initial cycle of selection, the level of survival gained from resistance can vary dramatically between tree species even in the same pathosystem and at the same level of improvement, whether MGR is present and whether virulence to MGR is present (e.g., Sniezko et al., 2020b). However, seed from the first cycle of testing and orchard development can offer resistance at a level that may be useful (e.g., >25% survival vs <5%). Results of the longer-term field trials will guide decisions for plantation forestry or restoration efforts where managers will have to decide what level of survival is sufficient and how to achieve it by increasing the number of seedlings to plant in the first cycle of selection. In some situations, managers may opt to wait for additional cycles of breeding to increase the level of survival among the seedlots used for planting.

2.2.2 Phase 2—Developmental: Seed production and breeding

The goal of a resistance program is to provide resistant planting stock for reforestation or restoration. Thus, the stock must be capable of surviving and reproducing under field conditions (prevailing disease pressure) in the target environments. In forestry, for most cases, seedlings (as opposed to vegetative propagules) will be used. In this case, seed may be collected from resistant parent trees in natural stands, or seed orchards that are established with grafts of resistant parent trees (output from Phase 1). In general, the seedlings from either of these two sources will be a diverse mixture of genotypes, with the average level of resistance being high enough to ensure land management objectives are met. Note that high levels of resistance in the initial screening cycle can be available, if MGR is present (e.g., Kinloch et al., 1970; Sniezko et al., 2014), but MGR is generally less durable than QR, especially if there is only one or very few major genes. Thus, the use of MGR could be precarious for many programs, even with noted exceptions (Amerson et al., 2015), but few, if any forest tree programs should utilize only MGR, unless there is evidence of its durability. The level of resistance achieved in the initial cycle of selection may be limited and will vary widely by species.

The level of resistance required by land managers will vary depending on the land management objectives. In the case of even-aged plantings, organizations may only be interested in tree species with very high levels of resistance and the highest probability for survival with few structural or stem defects. In other cases, land managers may be willing to plant extra seedlings (allowing for higher mortality in less resistant seedlings) to ensure objectives, such as maintaining the species as a component of a native forest ecosystem, will be accomplished. Resistance can be enhanced through breeding (crossing resistant parents), and then, progeny testing the parents can be identified in the first cycle of selection. Successive improvements in the average level of resistance can generally be expected in each generation of selection and crossing.

2.3 Biotechnology: Tools to accelerate an applied breeding program

Biotechnologies can assist applied breeding programs in three primary ways—clonal propagation, genetic marker analysis, and genetic engineering (Nelson, 2021). Clonal propagation is part of almost all applied programs, usually by grafting selected genotypes as parental stock for seed production or breeding (next-generation crossing). For some species, rooted cuttings can be used for the same purposes as well as for mass producing planting stock as a technology to supplement seed production. Advanced propagation technologies such as tissue culture, including micropropagation and somatic embryogenesis, can have important application as well but require advanced laboratory, growth chamber and greenhouse facilities and specialized expertise (e.g., Kong et al., 2014).

Genetic marker analysis is routine for many species and often integral to highly advanced applied breeding programs. The most common application is in validating genotype identity at various stages in the program with more advanced applications being genetic diversity analysis (e.g., Al-Rabab'ah and Williams, 2002; Eckert et al., 2010) and MAS, including genomic selection (e.g., Isik, 2014). It can be helpful to understand the genetic diversity of the species (tree and pathogen or pest) prior to initiating a program or early in its implementation. Making sure the genetic diversity of the species is well represented is important at the beginning of a breeding program, but even more important is measuring and monitoring diversity once resistant selections are made.

Genetic engineering involves recombinant DNA technology and can be viewed as a method to introduce or enhance resistance mechanisms into the species. Specialized facilities and advanced expertise are required for genetic engineering, in addition to a long lead time (typically decades or more) for developing the tissue culture and transformation systems (Harfouche et al., 2011; Chang et al., 2018) as well as a sequenced reference genome for the species. For commercial species, this work may be in development or even available to the applied breeding program. If so, the need for genetic engineering should be determined in Phase 1 of the program. If genetic engineering is determined necessary, candidate genes for various resistance mechanisms need to be evaluated, with the best candidates selected for use in the breeding program (Nelson, 2021). Following the transformation of genotypes from the breeding program, Phase 2 proceeds in much the same way, including early screening, field testing, seed production, and breeding of the transformed genotypes. Several possibilities and various considerations of using genetic engineering to advance forest health are discussed elsewhere (National Academies of Sciences, Engineering, and Medicine, 2019).

3. Case studies in forest tree resistance breeding

3.1 White pines and white pine blister rust

Nine five-needle white pine species (sub genus *Strobus*) are native in the United States with four of these being native to Canada (Tomback and Achuff, 2010). All nine species are highly susceptible to white pine blister rust (WPBR), caused by the non-native, invasive fungal pathogen *Cronartium ribicola* (Geils et al., 2010; Hoff et al., 1980; Sniezko et al., 2008) that has been present in North America since the early 1900s (Kinloch, 2003). More than 50 years ago, regional resistance breeding programs began for the three species of highest economic value: western white pine (*Pinus monticola*), sugar pine (*P. lambertiana*), and eastern white pine (*P. strobus*) (Bingham, 1983; King and Hunt, 2004; McDonald et al., 2004; Kriebel, 2004). The initial investigations discerned that genetic resistance was present in each of these species and that although relatively rare, it would be sufficient to develop (select and breed) populations of trees with resistance.

The USFS's regional program for the Pacific Northwest based at the Dorena Genetic Resource Center (DGRC) in Cottage Grove, Oregon, has been working on WPBR resistance consistently since it was established in 1966 (Fig. 10.3). The DGRC program has worked with all nine species of these white pines, to differing degrees, and a brief overview is provided here for four of them. The extensive early work with western white pine and sugar pine helped refine the seedling inoculation and evaluation protocols, as well as field trials, and this permitted relatively simple extension to the other species. The western white pine, sugar pine, and whitebark pine (*P. albicaulis*) programs have moved onto Phase 2, operational development of resistant populations, while the work in other species (other than eastern white pine, discussed below) is mostly focused on Phase 1, exploring levels and distribution of resistance to WPBR. Resistant seed of western white pine and sugar pine has been widely adopted by forest managers and used for decades with no evidence of negative impacts on growth or overall health of the improved planting stock that would suggest a negative correlations between resistance and other important traits.

The evaluation of resistance begins with tree selection and seed collection from parent trees (i.e., candidates for selection) in natural stands. In some cases, these candidates will be trees with little or no damage in stands with high mortality, while in other cases, these trees might be essentially random in proactive work to develop resistance (Johnson and Sniezko, 2021; Schoettle and Sniezko, 2007). Specific details on inoculation and seedling evaluation are presented

FIG. 10.3 (A) *Pinus albicaulis* (whitebark pine) at Crater Lake National Park (CRLA); (B) *P. albicaulis* at CRLA, infected with white pine blister rust (WPBR) caused by the non-native, fungal pathogen *Cronartiun ribicola*; (C) large inoculation "fog" chamber at Dorena Genetic Resource Center (DGRC); (D) an overview of white pine blister rust screening trials and greenhouse facilities at DGRC; (E) *P. albicaulis* screening trial for WPBR showing susceptible and resistant seedling families in 10-tree row plots; (F) sow year 2012 inoculation trial of *P. monticola* showing high surviving family with quantitative resistance; (G) recent field trial of *P. monticola* at BLM's Tyrrell orchard site; (H) older *P. monticola* trial at the Tyrrell site showing WPBR mortality in a family with major gene resistance, adjacent green trees have quantitative resistance; (I and J) WPBR cankers on *P. monticola* on trial at Washington Department of Natural Resource site—partners and cooperators in resistance program; (K) a restoration planting of *P. albicaulis* at CRLA—the planting is also a genetic trial with the parentage of each seedling known; and (L) breeding for resistance in a clone bank at DGRC, control crosses for *P. monticola*, conelets bagged to prevent insect damage. *Photographs: R. Sniezko.*

elsewhere (Kegley and Sniezko, 2004; Sniezko et al., 2011). In brief, seedling families from each candidate parent tree are generally grown for two growing seasons before commencing inoculation trials (Fig. 10.3C–F). Each trial generally includes progeny of 80 to 120 parent trees, with up to 60 seedlings per family (4800 to 7200 seedlings). Depending on the configuration of the trial (seedlings in containers or seedlings planted in raised boxes), the inoculation fog chamber at DGRC can accommodate 4800 to more than 10,000 seedlings at a time (Fig. 10.3C). After inoculation, usually in late August or September, seedlings are monitored for up to 5 years and evaluated for a range of resistance related traits (Kegley and Sniezko, 2004; Johnson and Sniezko, 2021; Sniezko et al., 2007, 2008, 2011, 2014).

The DGRC may have 50,000 or more seedlings on-site in different stages of evaluation in any year (Fig. 10.3D). The capacity to screen so many seedlings allows for finding the best families (i.e., candidate parents) and best individuals per family from several species simultaneously. The inoculation protocols have been refined in this system so that ~99% of seedlings show needle spots (first sign of infection), and the susceptible controls usually produce 100% mortality. Even with a controlled inoculum density, the number of needle spots or stem symptoms per seedling can vary from trial to trial or year to year. Thus, the inclusion of susceptible and resistant checklots is vital to help judge the efficacy of each inoculation trial. In western white pine and sugar pine, half-sib (open-pollinated) progeny of most field selections have less than 10% survival, emphasizing both the high susceptibility of these species and the efficacy of the inoculation system (Kegley and Sniezko, 2004; Sniezko et al., 2008). The plant material used for resistance screening could vary from half-sib families, full-sib families, self-pollinated families, or even rooted cuttings, but in this system, it is generally half-sib or full-sib families. In the first cycle, it can be faster or less expensive to utilize open-pollinated half-sib families obtained by making seed collections from candidate trees. This will provide information on the relative level of resistance between many candidate parent trees. At a later stage or as a separate component of the program, mating designs incorporating full-sib families can be used to further understand inheritance or produce families for advance-generation stages of the program. Full-sib families may be especially important when resistance levels are very low such that resistant x resistant full-sib families may be required to detect resistance.

To date, the DGRC has evaluated open-pollinated progeny from more than 4000 parent trees each for western white pine and sugar pine and from more than 1500 parents for whitebark pine. In addition, many more families derived from control crosses of western white pine and sugar pine candidate parents have also been evaluated in the first cycle of advance-generation breeding and beginnings of the second cycle. Although the system allows for large inoculation trials (Fig. 10.3C and D), the number of parents needed for testing (because resistance is rare), as well as variable annual budgets and seed supplies, has meant that several decades of trials have been needed to generate enough data to confidently select resistant parent trees for the various seed zones. Depending on funding, facilities and staffing, and knowledge gained from the most successful programs, the timeframe for similar projects with other systems could potentially be reduced dramatically.

In some five-needle white pine species, both MGR (i.e., qualitative resistance) and QR have been documented, while in others only QR has been found (Johnson and Sniezko, 2021; Kinloch and Dupper, 2002; Schoettle et al., 2014; Sniezko et al., 2008, 2014, 2020b). In the WPBR pathosystem, MGR results from a single dominant resistance gene (Kinloch and Dupper, 2002; Kinloch et al., 2003), while the inheritance of QR is more complicated and involves several traits (various measures of resistance), including ones that slow the progression of the rust (Johnson and Sniezko, 2021; Kegley and Sniezko, 2004; Sniezko et al., 2014). For western white pine and sugar pine, MGR is present in only portions of the species range, while QR appears to be more widely distributed (King et al., 2018; Kinloch et al., 2003, 2018). Only one major gene has been documented in each of these two species, denoted as *Cr2* in western white pine and *Cr1* in sugar pine. Evidence of evolved virulence by the pathogen, which renders MGR-based resistance ineffective, has been documented in both western white pine and sugar pine (Kinloch and Dupper, 2002; Kinloch et al., 2004; Sniezko et al., 2020b).

The level of QR varies between species, with whitebark pine showing much higher QR than western white pine, which in turn has a higher level than sugar pine. The levels of QR from initial field selections of western white pine and sugar pine are relatively low, with most families having less than 20% survival in testing (Kegley and Sniezko, 2004; King et al., 2018; Kinloch et al., 2018; Sniezko et al., 2008), necessitating the need for at least one cycle of breeding (through forward selections or resistant parent x resistant parent crossing) to increase resistance. Intensive breeding efforts have been underway for the last 15+ years and have generated some western white pine families with much higher levels of resistance than those in the original round of selection and testing (Fig. 10.3F and L) (Sniezko et al., 2014, 2020b). Field testing of a subset of the resistant and susceptible families has shown that the seedling screening assays are quite accurate, and results to date (more than 20 years in the field) suggest that the observed QR is durable and stable (Fig. 10.3G–J) (Sniezko et al., 2020b). Both QR and MGR selections have been retained in some seed orchards for western white pine and sugar pine. In contrast to western white pine and sugar pine, some seedling families of whitebark pine with QR show moderate-to-high levels of survival (40% to 90%) in seedling testing.

Selections, both reverse (parent-generation selections, based on progeny test performance) and forward (within-family selections from the progeny tests) are grafted and planted in seed orchards or a clone bank. Care must be taken, especially for seed orchards, to ensure potential inbreeding in the next generation is minimized. Often 10 or more years pass before enough pollen or seed cones are available for control crosses or operational seed production. Resistant seed from the first orchards is now available for western white pine and sugar pine and is being used in reforestation. No apparent negative correlations of resistance with growth or other traits have been noted. By contrast, in whitebark pine, the level of resistance is high enough in some seed zones that seed collected from parent trees in the wild is currently being used for restoration (Fig. 10.3K). However, cone crops of whitebark pine can be very sporadic and it can be very expensive to collect from scattered parent trees in remote locations (and these trees are subject to damage and mortality from fires and insects), so grafted seed orchards are also being established.

In contrast to the three species discussed above, trials to evaluate WPBR resistance in foxtail pine (*P. balfouriana*) only began in 2014. The first small trial of 21 open-pollinated families indicated that this species was perhaps the most susceptible of all the studied white pines, with 100% of seedlings cankered and only two of 705 seedlings surviving at the end of the test. More recent trials, still underway, are using lower inoculum densities to evaluate genetic resistance in a range-wide collection of 150 families. In addition, two field trials, established since 2020, will help confirm any resistance observed or missed in the screening trials. This first phase of testing should confirm whether this species is the most susceptible of the North American white pines but may also identify the first parent tree(s) with detectable levels of resistance. Although there are commonalities in approach to evaluating these four species for resistance to WPBR, experience has shown that each species has unique attributes that may require different inoculum densities or traits scored to quantify resistance.

For eastern white pine (*P. strobus*) in the Lake States, most of the early work with screening for WPBR resistance seemed to indicate that little resistance was present, but recent refinements indicate the early screening underestimated resistance (Pike et al., 2018). These new results revealed that 25 of 228 screened parents had resistance levels that equaled or surpassed the resistant standard. Grafted seed orchards have been initiated with these 25 unrelated genotypes, as have long-term field tests to evaluate the level of resistance under field conditions as well as the stability and durability of resistance. At the same time, additional candidate trees are being evaluated for resistance using the refined screening protocol in anticipation of adding diversity to the seed orchards and possibly finding higher levels of resistance.

Until recently, due to the large size of conifer genomes, relatively few genomic resources were available to aid in selection and breeding efforts. The recent efforts are starting to provide some information on the underlying genes and inheritance (Liu et al., 2021; Sniezko et al., 2014; Weiss et al., 2020) as well as tools for possible MAS (Liu et al., 2020). Tools for MAS could provide a more efficient method of making tree selections in the field, as well as combining both MGR and QR in seedling trial selections by allowing use of virulent strains of rust to expose seedlings with QR and the use of MAS to identify which of these seedlings also has MGR.

Dorena Genetic Resource Center and its facilities have been available for more than 50 years, with upgrades and expansions over time. Some of the technicians and staff (including the tree breeder) have been present for 20 or more years, providing continuity to keep the program functioning in a consistent manner. The extensive data collected over time on 1000s of parent trees and their progenies necessitates significant time be devoted to data management, and continuity of staff is helpful for this as well. Substantial progress has been made in developing resistant populations for several species; reforestation with WPBR-resistant seed is proceeding for western white pine, sugar pine, and whitebark pine; and further progress is expected from current breeding efforts.

3.2 Port-Orford-cedar and Port-Orford-cedar root disease

Port-Orford-cedar (POC; *Chamaecyparis lawsoniana*) is a long-lived conifer native to northwestern California and southwestern Oregon and is highly susceptible to POC root disease caused by *Phytophthora lateralis*, a non-native pathogen (Fig. 10.4) (Betlejewski et al., 2011). Prior to the accidental introduction of *P. lateralis* to the United States in the 1920s, POC had also been a popular species in urban plantings in both the United States and elsewhere (often referred to as Lawson's cypress). Early research inoculation trials seemed to suggest that there was no resistance in POC or that perhaps refinements in testing were needed, but resistance was eventually documented (Hansen et al., 1989). This stresses the need to take care in setting up artificial inoculation trials to ensure that they provide an accurate assessment regarding the variability in susceptibility/resistance within a tree species. Field plantings on diseased sites helped to confirm resistance at a relatively early stage and provided validation tests of early screening trials.

The confirmation of genetic resistance and concerns about the future viability of POC populations led to some further small-scale evaluations of resistance in Phase 1 and a decision by the USFS to begin Phase 2. This soon expanded to become an inter-regional program covering the entire geographic range of POC and involving the USFS's Pacific Northwest and Pacific Southwest Regions and the USDI Bureau of Land Management (BLM) (Sniezko et al., 2003,

FIG. 10.4 Resistance program for *Phytophthora lateralis* in Port-Orford-cedar (POC; *Chamaecyparis lawsoniana*) (A–C) dead and dying trees in Redwood National Park, California and southern Oregon, (D) resistant parent "510015" surrounded by POC trees killed by *P. lateralis*, (E and F) raised bed testing of seedling families, (G and H) root dip inoculation testing of seedling families, (I) containerized breeding orchards, (J) field trial under dead POC at South Slough National Estuarine Research Reserve, (K) clone bank at BLM's Tyrrell orchard site, (L) restoration trial in southern Oregon planted by volunteer groups. Refer to Fig. 10.2 for the general components of the resistance breeding program. *Photographs: Chuck Frank (D), Scott Kolpak (L), and Richard Sniezko (A, B, C, E, F, G, H, I, J, K).*

2012a). The program has developed very rapidly due to commitments to test over 5000 candidate parent trees in the first 2 years (and many more in subsequent years), as well as the favorable biology of the species which includes ease of clonal propagation (rooted cuttings) and sexual reproduction (early flowering in potted plants) (Fig. 10.4I) (Elliott and Sniezko, 2000). In addition, a long-term partnership with Oregon State University (OSU) has been established to screen these materials (rooted cuttings or seedling families of the parent trees) for resistance (Hansen et al., 2012).

Both MGR and QR have now been documented in POC (Sniezko et al., 2020a), and results from the first field trials provide early, first confirmation of the utility of the seedling root dip test (Sniezko et al., 2012b). Note that several types of seedling screening protocols have been tried, including (a) a stem dip test, (b) a root dip test (using seedlings or rooted cuttings), (c) a raised bed test (using seedlings or rooted cuttings), and (d) field trials with seedlings or rooted cuttings (Hansen et al., 2012). The use of a root dip test of seedling families seems to provide the best, most consistent resolution among families (Sniezko et al., 2020a), and good correspondence with field results (Sniezko et al., 2012a). Extending the observation period from 1 to 3 years provided the additional data necessary to determine that both MGR and QR were present (Sniezko et al., 2020b). Seed from half-sibs, full-sibs, and self-pollinations have been used in testing, with the majority of testing now done using seed from self-pollinations (Sniezko et al., 2020a). For POC, self-pollination is operationally much easier than creating outcrossed full-sibs, even with some reduction in seed yield, since a pollination bag can easily enclose many pollen cones and seed cones in the same bag.

Root dip trials can be relatively large, including 100 or more families with 42 seedlings per family, and several trials can be initiated in a year. Results from these trials (progeny tests) are used to decide on which parents to select and place into containerized seed orchards (Fig. 10.2). In addition, forward selections, usually surviving seedlings in the better families, are made from the initial trials and placed into containerized seed and breeding orchards. Rooted cuttings are used to propagate the selected genotypes for the containerized orchards which are maintained in unheated greenhouses (Fig. 10.4I). Seed production from GA_3-treated trees in the orchards can be prolific providing ample seed for many of the targeted reforestation sites in the 13 breeding zones delineated for this species.

Field trials have been established (Fig. 10.4J and L) and will continue to be needed to validate resistance and its durability over the long term. In this species, the first resistant seed was available for some breeding zones within 5 years of program inception. As future testing is completed, the number of plants at DGRC will be reduced greatly, which will reduce the ongoing cost of the program accordingly. For at least some breeding zones, resistance may be high enough after the current cycle of selection and breeding to stop at this stage. A clone bank was also established in a BLM seed orchard complex and serves to provide a backup to genetic material in the containerized orchards (Fig. 10.4K). Containerized seed orchards have been started for most breeding zones, and there are currently >50 clones in several of these, with potential to increase the number of parents further as testing proceeds. The ability to manage POC in containerized orchards greatly simplifies the upgrading of orchards. Current tree improvement efforts are aiming to increase the number of resistant parent trees available for each breeding zone and continue breeding efforts to increase resistance. Also, initial efforts are being made to examine the need for different orchards for serpentine versus non-serpentine soils. Resistant seed is now available and being used by an array of organizations in the zones of highest disease pressure.

The progress made in the POC resistance program and the subsequent use of the seed have been noted by the International Union of Conservation of Nature (IUCN Red List), which had listed the species as "vulnerable" in 2000, but downgraded its status to "near threatened" in 2013, with the expectation that it will be listed as a species of "least concern" within 10 years if current conservation actions, including planting resistant seedlings are successful and maintained (Farjon, 2013). The combination of the infrastructure and staff provided by the DGRC along with pathology expertise and support provided by OSU and USFS, and support from USFS and BLM silviculturists and other professionals and technicians greatly aided the progress with this species. The demand for POC seed from the DGRC orchards by various organizations has increased in the last few years as word of the availability of resistant seed has become known.

At this stage, there has been little development of genomic resources for POC, but with the large populations available at DGRC and well-characterized parental genotypes, there is ample opportunity to proceed, and this would provide further insights into inheritance of resistance and its potential durability. Since its inception, the resistance program results have been presented at periodic POC technical committee meetings and scientific conferences to provide information to stakeholders and receive inputs to help continue progress.

3.3 Loblolly pine and fusiform rust

A classic example of resistance breeding in tree species with high economic value is provided by the southern US pines (primarily loblolly pine, *Pinus taeda*, and slash pine, *Pinus elliottii*) and fusiform rust, caused by the fungal pathogen, *Cronartium quercuum f.* sp. *fusiforme*. In contrast to the previous two examples, this is a case of native tree species being affected by a native pathogen. Recent and older papers have highlighted specific aspects of this success (McKeand et al., 2003; Schmidt, 2003; Walker and McKeand, 2018), while other papers have highlighted advances in the genetics and pathology of the pathosystem (Powers et al., 1981; Wilcox et al., 1996; Kubisiak et al., 2005; Amerson et al., 2015). Clearly, the operational development of the loblolly and slash pine breeding programs in parallel with basic research activities has contributed to the remarkable success of the effort where highly rust-resistant planting stock can be readily purchased at scale for planting in any area of the southeast United States (Pye et al., 1997; Cubbage et al., 2000; McKeand et al., 2003;

McKeand, 2019; McKeand and Bridgwater, 1998). At the same time, it is important to point out that progress in developing resistant pine populations was made early on despite uncertainties about the specifics of the genetic control of resistance. A clearer understanding of the underlying control of resistance (e.g., at least nine major genes) has only recently been elucidated (Amerson et al., 2015), and work continues to refine our understanding of this pathosystem (Lauer and Isik, 2021; Ence et al., 2021). As a native pathogen affecting a native species, an understanding of the geographic distribution of resistance and virulence provides insights into durable and stable resistance in a natural pathosystem that can inform deployment decisions in managed pine plantations (Nelson et al., 2010).

The fusiform rust resistance programs were initiated and implemented largely using the framework described in the previous examples and Fig. 10.2. The exploratory and developmental phases largely overlapped each other which are not atypical for a native pathogen or pest. In this case, infrastructure and protocols for screening were developed as sources of genetic resistance and candidate trees were being identified (Jewell, 1960; Jewell and Mallett, 1974; Barber et al., 1957; Goddard and Schmidt, 1971; Kinloch and Stonecypher, 1969). Phase 2, essentially scaling up the initial phase, began in earnest in the 1970s and continued into the 1980s (Matthews and Rowan, 1972; Snow and Kais, 1972; Powers et al., 1981; Schmidt et al., 1981, 1986). By the 1990s, the program was mature and largely steady state in terms of bringing on new sources of resistance in the form of advanced-generation selections or new plantation selections, and continuing to improve all program components, as well as increasing understanding of the basic biology of the host-pathogen interaction and operational guidelines for utilizing rust-resistant planting stock (McKeand et al., 1989; Isik et al., 2003, 2008, 2012; Bridgwater et al., 2005).

The Resistance Screening Center (RSC, Cowling and Young, 2013) has been and continues to be instrumental in the success of early screening, while the longer-term progeny tests are conducted by regional tree improvement cooperatives (i.e., NC State University Tree Improvement Program, Western Gulf Cooperative Forest Tree Improvement Program through Texas A&M University, and Cooperative Forest Genetics Research Program through the University of Florida). The progeny tests are highly refined and provide accurate and timely information on tree growth and form and fusiform rust resistance for use in advanced generation breeding and deployment through seed orchard seed (e.g., Hodge et al., 1990; Brawner et al., 1999; Xiang et al., 2003; Vergara et al., 2007). The scale of the screening and field-testing programs ensures that high-quality information of the durability and stability of resistance is continually monitored and updated (McKeand et al., 1999; Gramacho et al., 2013; Kubisiak et al., 2004; Hodge et al., 1993; Schmidtling and Nelson, 1996). In each cycle, resistance is improved in the orchards and among the parents used in breeding to provide the next generation of selection candidates.

Biotechnology has aided this effort through vegetative propagation, mostly cloning parent trees by grafting, as well as through genetic marker studies. The genetic marker studies have assisted breeders in tracking their materials through the breeding and deployment activities and researchers in understanding the nature of resistance and the host-pathogen interaction (Echt et al., 2011; Kubisiak et al., 2011; Resende Jr. et al., 2012; Quesada et al., 2014; Nelson et al., 2010; Amerson et al., 2015; Pendleton et al., 2014). Continued work on mapping resistance and virulence genes in the genomes of the applied programs' parent trees (genetic/genomic mapping) and on the landscape (landscape host/pathogen genomics) will further improve the level of resistance achieved in the planted forests and leverage the breeding program's past and ongoing investments. Recent updates to the loblolly pine reference genetic map (Westbrook et al., 2015) and the development of a draft genome sequence (Neale et al., 2014) will continue to facilitate work driving at developing breeding tools such as MAS and genomic selection.

4. Summary: Outlook for the future

Resistance breeding programs will be essential for society if we are to retain the utility of many of our forest tree species that are seriously affected by diseases or pests. However, well-designed and well-focused programs are needed, and this is where many efforts come up short or fail all together (Nelson and Koch, 2017). We have discussed the two distinct phases of resistance breeding and argue that putting these phases in practice from the beginning will be instrumental to success. Durability (resistance over decades) and stability (resistance over varied environments) will be key for forest trees, and there is cautious optimism that this will generally be the case for many current programs (Sniezko and Liu, 2021). Fortunately, successful forest tree resistance programs and guidance from their extensive experience can be utilized to help ensure the success of new programs. The three examples discussed here show that substantial progress in resistance can be made through traditional breeding/tree improvement, even without a solid understanding of the underlying genetic-based mechanisms. For example, a relatively recent program designed to develop koa wilt resistance in *Acacia koa* in Hawaii capitalized on the framework outlined here and collaboration with the DGRC to develop resistant seed in less than 15 years (Dudley et al., 2020). In addition, an incipient program for developing resistance to Rapid ʻŌhiʻa Death (ROD) in Hawaii is doing likewise, as well as assembling a diverse team to assist in program development and implementation (Luiz et al., 2022).

A successful resistance breeding program will include many forest genetic and tree improvement concepts and components, details of which are beyond the scope of this chapter. In some cases, a disease (or pest) resistance initiative will be included within an existing tree improvement program. Programs such as those to develop fusiform rust resistance in loblolly and slash pines in the southern United States already house the basic tree improvement infrastructure needed to incorporate a novel trait such as disease resistance. However, in other cases, a resistance program is necessitated without experienced staff, supportive infrastructure in tree improvement, or any prior genetics knowledge of the host tree species. Fortunately, several very good textbooks relating to forest genetics, tree improvement, and some considerations for selection and breeding for resistance to diseases, pests, or adverse environments (White et al., 2007; Zobel and Talbert, 1984) are available to offer additional guidance to program managers and new tree breeders. In addition, studying the current successful forest tree programs (e.g., Sniezko and Koch, 2017; McKeand, 2015) at the onset of a new program would be invaluable. In addition, several universities offer in-place or online courses in tree breeding and additional offerings in this arena would be useful.

Resistance breeding programs are simplest to undertake when tree improvement facilities and experienced staff are already in place. Unfortunately, over the last few decades, the number of tree improvement programs and experienced tree breeders has declined significantly (Wheeler et al., 2015). However, there is growing recognition of the need for resistance tree breeding programs (Nelson and Koch, 2017; Federman and Zankowski, 2020; National Academies and of Sciences, Engineering, and Medicine., 2019; Pike et al., 2021), and if this translates into added resources, then there will be ample opportunities to initiate and implement new programs. In addition, biotechnological advances (e.g., MAS, high-throughput genotyping and phenotyping, genetic engineering, and genome editing) have the potential to increase the efficiency and efficacy of resistance breeding. The biological potential to develop resistant populations for any forest tree species affected by disease or pest is present and waiting to be discovered and advanced to improve forest health.

5. Study questions

(1) How will you determine whether resistance is durable?
(2) How will you determine whether the resistance is major gene or quantitative, and why does it matter?
(3) If more than two species are susceptible to the same pathogen, how much might you expect them to be similar or different in the level and frequency of resistance?
(4) In what cases will you need to go beyond the initial generation of resistance testing and selecting and breed one or more cycles for resistance?
(5) When might biotechnology (MAS or genetic engineering) be useful in a tree resistance program?

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