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Breeding Roses for Disease Resistance

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I. INTRODUCTION

The cultivated rose (*Rosa hybrida* L., Rosaceae) is arguably the world's most famous ornamental plant. Roses are utilized in the florist industry as both cut stems and potted specimens. Garden roses such as hybrid teas, shrubs, polyanthas, and other types with various flower colors and forms

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fit a wide range of landscape niches. Rose hips possess high levels of vitamin C and cancer-preventing compounds in addition to their ornamental attributes (Wen et al. 2006). Even rose prickles have visual beauty in their diverse colors and types. As a result, roses are also the world's most economically important ornamental crop. As of 2003, 8 billion cut stems, 80 million potted specimens, and 220 million landscape roses were sold annually (Roberts et al. 2003). In the United States alone in 2005, the wholesale value of cut flower roses was $40 million, with the wholesale value of imports reaching $277 million (USDA 2006). Low-maintenance roses for landscape use is one market that is growing in popularity since roses bloom over an extended period and great diversity can be found for traits such as flower color, plant habit, and fragrance (Lonnee 2005).

Disease susceptibility poses the greatest challenge for producing and maintaining quality roses for all market niches but especially low-maintenance landscape types. A litany of microbes are pathogenic on roses, several of which are capable of serious damage. Black spot disease, caused by *Diplocarpon rosae* Wolf, is considered the most severe disease of landscape roses due to the potential for quick defoliation leading to compromised plant health and unsightly appearance (Dobbs 1984). Powdery mildew of roses, caused by *Podosphaera pannosa* (Wallr.: Fr.) de Bary, is another significant and widespread disease of roses that causes damage in landscapes and especially in greenhouses (Horst 1983).

The general disease susceptibility of cultivated rose, combined with its importance as an ornamental plant, makes host resistance a worthy breeding goal. Furthermore, concerns over pesticide use in general also motivate the search for more natural methods of disease control. Development of durable genetic resistance as a control strategy should prove to be more efficient and environmentally sustainable than chemical pesticide application. Therefore, substantial research has been conducted in recent years using new molecular tools to elucidate the biology of rose pathogens, develop methods for resistance screening, and devise strategies for resistance breeding.

Here we examine the extant literature on the biology and life history of the major pathogens causing disease on rose including fungi, bacteria, nematodes, and viruses. The review focuses on the pathogens causing black spot and powdery mildew diseases due to their primary significance. We then review efforts to screen for resistance genes in roses, the utilization of genes in breeding efforts, and the use of molecular approaches such as markers, candidate genes, and genetic transformation. Finally, we discuss the future prospects for breeding and development of disease-resistant roses.

II. CAUSAL PATHOGENS

A. Black Spot Disease

Rose black spot is caused by an infection of the fungus *Diplocarpon rosae* (*Marssonina rosae* anamorph), an ascomycete fungus in the Dermateaceae. This organism is obligate to the genus *Rosa* and thus does not infect any other plant taxa. *D. rosae* is hemibiotrophic, which means that it is parasitic on living host tissue but also has limited ability for saprophytic growth (Blechert and Debener 2005).

Diplocarpon rosae is mainly spread through asexual spores called conidia. Conidia are produced in abundance from acervuli within infection sites on leaves and stems. These conidia overwinter on dormant stems and fallen leaves until they are disseminated to new growth in the spring via water splash (Horst 1983). Although the younger, upper leaves are more susceptible than older leaves, the lower leaves usually are infected first, presumably because they are most affected by water splash. Free water is necessary for the fungus to germinate and directly penetrate the epidermis of rose leaves and stems, even when humidity is 100% (Horst 1983). On susceptible rose genotypes, subcuticular hyphae radiate from the infection site followed by branching intercellular hyphae that give rise to intracellular haustoria (Blechert and Debener 2005). Symptoms may appear to the naked eye in as little as 4 days from infection, with acervuli rupturing the leaf surface in 10 to 14 days (Horst 1983). As lesions expand in size, characteristic yellowing of leaf tissue occurs and is soon followed by defoliation. The damage to plant health and appearance that results from defoliation is what makes black spot the most serious disease of roses in the outdoor landscape (Dobbs 1984).

There are challenges to culturing and storing *D. rosae* that are pertinent to breeders who wish to perform controlled inoculation experiments. Fungal hyphae grow slowly across culture media, and spores produced in culture may not be virulent. To ensure the accuracy of inoculation tests, care must be taken to maintain pathogenicity when storing isolates long term and when growing inoculum in culture plates. The fungus is commonly isolated and cultured on potato dextrose agar (PDA), yeast malt extract agar (YMEA), and biomalt media. However, cultures begin to lose pathogenicity after six months at 23°C (Drewes-Alvarez 1992). Refrigeration does not solve this problem, as cultures stored at 1°C to 2°C declined gradually and lost all infectivity after 13 months (Palmer et al. 1996b). Researchers have resorted to maintaining single-spore isolates by freezing infected leaf pieces. Results at −15°C to −20°C are better than at 1°C to 2°C, with conidia on leaf surfaces

remaining viable for a year (Knight and Wheeler 1978a; Yokoya et al. 2000; Leus 2005). Yet cryogenic storage appears to be the most effective method for preserving black spot isolates long term. Castledine et al. (1982) succeeded in freezing conidia for 24 hours in liquid nitrogen (−196°C) with no loss of viability. Subsequently, Whitaker et al. (2007b) successfully stored infected leaf pieces for several months both in the gas phase of liquid nitrogen and at −80°C. These low-temperature treatments were superior compared to −20°C for three different isolates. Conidia harvested from culture plates have also been stored in glass beads above liquid nitrogen (Carlson-Nilsson 2002), although the effectiveness of this method has not been reported.

It is advisable to periodically reinfect isolates to susceptible rose cultivars in order to maintain pathogenicity. Spencer and Wood (1992a) bypassed long-term storage strategies altogether by reinoculating isolates to greenhouse-grown roses every 2 to 3 months. Walker et al. (1996) found that conidia grown in culture on MEA did not infect leaf discs excised from 'Frensham' rose leaves grown in vitro, even when the leaf surface was mildly abraded. Yet the same conidia successfully infected ex vitro grown 'Frensham' leaves at an average rate of 61.7%. The conidia produced on these ex vitro leaves were then capable of infecting 'Frensham' leaves grown in vitro. The authors suggested that conidia grown in culture may have been less virulent. Based on these results, the authors subsequently stored all isolates on ex vitro leaves. Evidently, a totally in vitro system is not effective for maintaining virulent *D. rosae* isolates.

Diplocarpon rosae appears to be a diverse species, as indicated by phenotypic observations and genetic studies. Conidial morphology and colony color are quite variable among isolates when grown in culture (Wenefrida and Spencer 1993; Whitaker et al. 2007b). Genetic analysis of the internal transcribed spacer (ITS) region of rDNA by restriction fragment length polymorphism (RFLP) analysis separated 10 *D. rosae* isolates into three groups with distinct RFLP patterns (Lee et al. 2000). Preliminary studies by British researchers have revealed genetic diversity in a collection of isolates by using simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers, concluding that AFLP was a sensitive and reproducible marker system appropriate for *D. rosae* (Drewes-Alvarez 2003). AFLP analysis of 50 isolates collected from 14 locations throughout eastern North America revealed significant diversity among isolates (Fig. 6.1). Over 20% of AFLP fragments were polymorphic, reflecting a higher level of diversity than has been found in AFLP studies of some other fungi in the Dermataceae family, including *Pyrenopeziza brassicae*

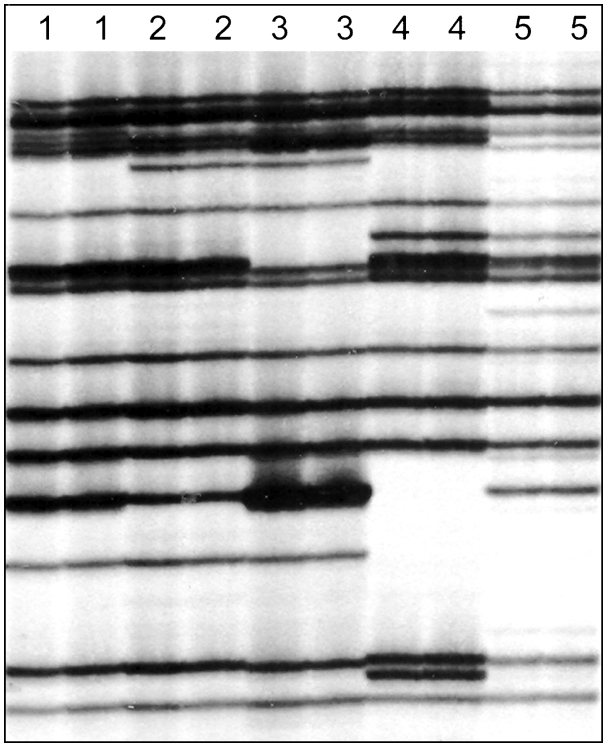

Fig. 6.1. Polymorphisms between five different *Diplocarpon rosae* single spore isolates collected from eastern North America, as revealed by AFLP. Pairs of lanes for each isolate represent duplicate selective amplifications.

(pathogenic on crucifers) and *Tapesia yallundae* (pathogenic on wheat). Analysis of the marker data for *D. rosae* showed considerable diversity among isolates within each collection site and did not reveal any clustering of isolates based on geographic location (Whitaker et al. 2007a). It is possible that the commercial distribution of roses throughout North America could contribute to this phenomenon, through the movement of *D. rosae* on infected vegetative propagules. The results suggest that screening for resistance in one location could be as effective as screening in multiple locations in this region. However, field screening within even a single location is problematic because of uneven disease progression due to environmental factors and nonuniform distribution of pathogenic races. This problem can be avoided by artificially inoculating with known races in the greenhouse and lab.

Although the diversity of *D. rosae* does not appear to be geographically structured in eastern North America, a random amplified polymorphic DNA (RAPD) analysis of 15 isolates collected from locations in Canada, France, Sweden, and the United States exhibited some clustering based on geography (Carlsson-Nilsson 2002). It is not known to what extent sexual recombination contributes to diversity, but the sexual stage of *D. rosae* has been observed only twice in England and twice in North America (Horst 1983). Conclusions about sexual recombination based on these limited observations should be guarded, since recent genetic studies have revealed recombination in several apparently asexual species of fungi in the Ascomycota (Taylor et al. 1999).

Genetic diversity of *D. rosae* is also reflected in a diversity of pathogenic races. Races are determined by inoculating multiple isolates to a collection of host genotypes. Isolates that can be differentiated from one another based on their host range are called races or pathotypes. In 1977, the previously resistant rugosa rose cultivar 'Martin Frobisher' suddenly became infected with black spot (Bolton and Svejda 1979). Further inoculation tests in Ontario, Canada, revealed the presence of four different races of the fungus (Svejda and Bolton 1980). In 1992, seven races were discovered in a small, seven-county area in Mississippi (Spencer and Wood 1992a, 1992b). Meanwhile, five races were discovered in Germany (Debener et al. 1998). Four races were differentiated in Belgium (Leus 2005), and four races were described in England (Yokoya et al. 2000). Recently three races were differentiated from among 14 isolates collected in eastern North America (Whitaker et al. 2007c). These three races are the only described races from North America that are known to be maintained long term in a virulent state. The loss of other collections is presumably due to the aforementioned difficulties in storing this fungal species. Pathogenic races described in Europe and North America have not yet been compared, although a standard host differential set is currently being established.

B. Powdery Mildew Disease

Powdery mildew fungi are classified in the family Erysiphaceae, in the class Ascomycetes. The Erysiphaceae is comprised of 13 genera and approximately 650 species, although detailed studies utilizing modern techniques have not been applied systematically within the family (Braun et al. 2002). Powdery mildews are obligate, biotrophic fungi, meaning they can survive only on cells in specific living hosts. Despite their restrictive host specificity, powdery mildews are ubiquitous,

infecting all crop plants worldwide with the exception of pines and their relatives (Horst 1999).

Most powdery mildew infections are restricted to the epidermal surface, appearing as masses of white hyphae that exist in mats on the leaf surface (Bushnell 2002). Hyphae are attached to the leaf by pegs that penetrate the epidermal cell walls. At these attachment points, haustoria form, which allow the fungus to absorb nutrients from the leaf tissue. The characteristic powdery white appearance associated with a powdery mildew infection results from vegetative hyphae or conidiophores. The hyphae grow out from the leaf and produce chains of asexual spores or conidia. Sexual spores occasionally are produced in spherical structures, ascomata, which appear as reddish-brown dots in the hyphal mats.

Powdery mildew disease of rose is incited by an infection of the fungal organism *Podosphaera pannosa* (Wallr.: Fr.) de Bary. Prior to revisions in the generic system (Braun and Takamatsu 2000), the organism was known as *Sphaerotheca pannosa* var. *rosae* (Wallr.: Fr.) Lév., which was differentiated from *S. pannosa* var. *persicae* Woron., a *Prunus*-infecting isolate. Morphological and DNA sequence data did not differentiate the two species (Takamatsu et al. 1998; Saenz and Taylor 1999), and they were subsequently considered to be a single species that is capable of infecting rose and *Prunus* species. Powdery mildew disease of rose is the most widely occurring disease of rose worldwide (Horst 1983). While occurring on garden-grown roses, the disease occurs most frequently and has the largest impact on greenhouse-grown roses (Linde and Shishkoff 2003). Powdery mildews propagate primarily through vegetative means, via asexual spores or conidia. In the outdoor environment, infections occur in autumn on dormant buds where the pathogen overwinters. When plant growth resumes the following spring, the fungus on the newly emergent foliage produces conidia that are dispersed via wind. Conidia land on newly emergent plant tissue. Germination and initial development of the fungus is enhanced by elevated humidity but impeded by the presence of free water (Sivapalan 1993).

In the greenhouse, infections generally begin with the introduction of infected plants or via conidia through vents, fans, insects, human hands, or clothing. Infections spread rapidly in the highly conducive environment created in the greenhouse. Once an infection of powdery mildew is established in a greenhouse, it is difficult to eradicate. An eradication effort typically entails disposal of all rose plants, settling of all conidia, disinfecting all greenhouse surfaces, and establishment of a more rigorous prevention protocol.

Conidia germinate optimally (within 2 to 6 hours) at a temperature of 23°C and 100% humidity (Xu 1999). Temperatures above 30°C were reported to prevent germination (Xu 1999). In humid conditions, conidia can survive for up to 3 months at low temperatures, 0°C to 3°C (Price 1970). Conidia germination and fungal growth can occur in relative humidity as low as 50%, although at decreased levels (Rogers 1959; Pathak and Chorin 1969). Other environmental factors that promote powdery mildew infection and disease progression include shade, closely spaced plantings, and profuse foliage growth, all of which serve to reduce air circulation and/or promote increased humidity (Linde and Shishkoff 2003). In optimal conditions conidia can germinate, colonize, and produce new conidia in 4 to 10 days (Linde and Shiskoff 2003).

The fungus is capable of producing sexual fruiting bodies in late summer if both mating types are present. The fruit bodies called ascocarps are formed by the sex organs of the fungus, the gametangium and the ascogonium, on lateral branches of the mycelium. The ascocarps contain 4 to 8 ascospores that are capable of infecting young rose tissue. In field observations reported by Price (1970), ascocarps were rare and were found in only ~5% of cases. However, cleistocarps (equivalently termed ascocarps) were reported in over 700 rose species and cultivars inspected over three seasons (Price 1970). The presence of ascocarps offers the opportunity for genetic recombination and the formation of new races of the pathogen.

After germination, an appressoria develops at the end of the germination tube, which attaches the mycelium to the rose plant surface by a fine slime layer (Hajlaoui et al. 1991). A penetration peg emerges through a pore in the appressorium and enters the cuticle and underlying epidermal cell wall. In the epidermal cell, the penetration peg enlarges to form the haustorial neck. From the center of the attachment of the appressoria, multilobed, globose mature haustoria are formed (Hajlaoui et al. 1991). The haustoria serve to absorb nutrients for the fungus from the rose host. Haustoria continue to form as hyphae extend along the leaf surface.

In addition to the environmental factors just noted, symptoms of powdery mildew infection in rose also can be influenced by the host tissue infected, age of the tissue infected, as well as host genotype. Rose leaves are most readily infected in the first 3 days after unfurling (Linde and Shishkoff 2003). Infections at this stage typically result in twisted, stunted, or distorted leaves. Powdery mildew can spread quickly on young leaves, leading to shriveling and defoliation. Infections on young leaves often result in dark reddish spotting on

the leaf before appearance of the characteristic powdery white hyphal growth. Older leaves are less susceptible and infection progression is slower, often resulting in a patchy occurrence of the fungus.

Young rapidly extending stem tissue can become infected, often where a thorn attaches. The infection generally will persist as the stem matures, resulting in irregular powdery patches of fungus on the stem. Fungal infections also can occur on the flowers. Generally infections start on unopened flowers on the calyx of unopened flower buds. The infection often spreads to the petals as the flowers open, leading to distorted, poor-quality flowers (Horst 1983).

Numerous claims of powdery mildew resistance for specific cultivars are made in popular and nursery trade literature, but durable resistance to the disease is seldom seen. Initially, inoculations and observations of rose cultivars and species revealed little difference between species and few cultivars with broad resistance (Mence and Hildebrandt 1966; Atkiss 1978). Subsequent inoculations with single conidial isolates have confirmed the presence of multiple races of powdery mildew (Bender and Coyier 1984; Linde and Debener 2003), but not all tests have differentiated races (Leus et al. 2002, 2005).

C. Other Diseases

Other fungal diseases include, but are not limited to, botrytis blight caused by *Botrytis cinerea Pers*. ex Fr., Cercospora leaf spot caused by *Cercospora puderi* B.H. Davis and *Cercospora rosicola* Pass., downy mildew caused by *Peronospora sparsa* Berk., rust caused by various species of the genus *Phragmidium*, and spot anthracnose caused by *Sphaceloma rosarum* (Pass.) Jenkins (Horst 1983). Botrytis blight is an important disease of many crop plants. It is significant on roses mainly because of its detrimental effect on flower bud appearance, although it also can cause blighting of canes (Horst 1983). *Botrytis cinerea* is biologically amenable to controlled inoculation experiments. Isolates can be grown easily and maintained in culture, but loss of pathogenicity is known to occur and should be considered (Pie and Brower 1993). Cut roses often are infected during storage and shipping because of cool, moist conditions. Optimum conidial germination of occurs at 15°C, and lesions appear on petals as early as 8 to 12 hours postinoculation when relative humidity is maintained at 100% (Horst 1983; Pie and Brouwer 1993). Finding genetic resistance is increasingly important, since populations of *B. cinerea* have developed resistance to fungicides (Gullino and Garibaldi 1996).

Downy mildew causes few problems in low-humidity environments, but under cool conditions with humidity greater than 85%, leaf abscission can be more serious than for black spot disease (Horst 1983). Downy mildew symptoms include purplish or reddish-brown irregular spots on the upper leaf surface, with mycelia and conidial growth occurring on the lower leaf surface. Free water or high humidity is required for spore germination, with spores germinating at a high rate between 2°C and 18°C (Xu and Pettitt 2003). Conidia may be stored in frozen water suspensions (15% DMSO) for 6 months at −20°C without complete loss of virulence (Schulz and Debener 2005). Opinions differ as to whether *P. sparsa* overwinters as mycelia on or within stem tissue. Xu and Pettitt (2003) report that oospores appear to be the main source of overwintering inoculum and that winter sanitation of diseased leaves is an effective control measure. However, polymerase chain reaction (PCR) and microscopic assays as well as fungicide trials have confirmed the presence of perennial infections of *P. sparsa* within rose stem tissue (Aegerter et al. 2002). For this reason, chemical treatment and/or sanitation of propagation source plants may be necessary.

Spot anthracnose is continually spread by water-borne conidia during the summer months. Occasionally its incidence can be as serious as a black spot infestation under humid conditions. Spot anthracnose is commonly mistaken for black spot but can be visually distinguished by the purplish cast of young lesions, the centers of which ultimately turn gray or white and fall away, leaving a shot-hole appearance (Horst 1983). Ethylene production is increased in rose leaflets infected with this disease (McClellan 1953). Other than this, little information has been published on the biology of this disease, which is surprising considering its prevalence.

Crown gall is caused by the bacteria *Agrobacterium tumefaciens* Conn., which typically enters the plant through stem wounds. Infection results in masses of undifferentiated tissue that can reach several centimeters in diameter on susceptible roses, resulting in serious damage if located at the root-shoot junction (Horst 1983). *Agrobacterium tumefaciens* can remain latent in rose tissues at temperatures below 10°C but will remain viable and cause symptoms when higher temperatures are reached. Wounding of stem tissue should be minimized during cultivation and handling, and pruning tools should be disinfected to prevent spread (Gullino and Garibaldi 1996). Isolation from roses can be performed by macerating galls and growing the gall extract on peptone-yeast-glucose agar (LPGA) medium (Pionnat et al. 1996). However, not all isolated strains can cause tumors on rose.

Only strains containing the Ti plasmid, a circular strand of DNA, are pathogenic. The Ti plasmid contains two elements necessary for tumorigenesis, the transferred DNA (T-DNA) and *vir* genes. The T-DNA, which encodes plant growth hormones and other compounds, is transferred into the plant cell nucleus and incorporated into the host genome. The *vir* genes mediate the transfer and incorporation process (Sheng and Citovsky 1996). Therefore, virulent strains may be detected by confirming the presence of the Ti plasmid. This has been accomplished in roses with PCR, using primers specific to portions of the *vir* genes and T-DNA (Pionnat et al. 1996).

Nematodes pathogenic to roses are distributed worldwide and can cause symptoms of dwarfing, reduced vigor, wilting, and chlorosis. Horst (1983) lists an astounding 18 genera that have been found on roses. The two most important species infecting roses are *Meloidogyne hapla* Chitwood (root-knot nematode) and *Pratylenchus vulnus* Allen and Jensen (root-lesion nematode) (Voisin et al. 1996). Races of *M. hapla* have been reported based on an inoculation of isolates obtained from Canada and France to *Rosa indica* (Wang et al. 2004). Nematode infestation is most problematic in field production nurseries. Methyl bromide fumigation of the soil has been an effective control measure in the past. However, this chemical is being phased out by law because of its harmful environmental effects, and alternative fumigants currently are being tested for their effectiveness (Schneider et al. 2005).

Like nematodes, viruses can cause symptoms of stunting, chlorosis, and general decline. Though there is no mandatory virus-free certification program, the Foundation Plant Services (FPS) at the University of California, Davis, and Florida Southern College provide virus-tested material (Manners 1993). By rule, new All-America Rose Selections winners are required to enter the FPS program (Foundation Plant Services 2007). At least 11 different virus diseases are reviewed by Horst (1983). Among the important causal viruses affecting rose are rose mosaic virus (RMV), strawberry latent ringpot virus (SLRV), and rose streak virus (RSV). A previously undescribed filamentous virus of roses, preliminarily named rose yellow mosaic virus (RoYMV), was discovered in 2004. Rose yellow mosaic virus causes severe yellow sectoring and mosaic symptoms on leaves and necrotic ringspots on the stems of at least one genotype (Fig. 6.2). Nucleotide and amino acid sequence comparisons to previously described viruses yielded no close matches (Lockhart and Olszewski 2007). For purposes of breeding, development of robust methods for controlled inoculation of the major viruses and rating of symptoms would be beneficial.

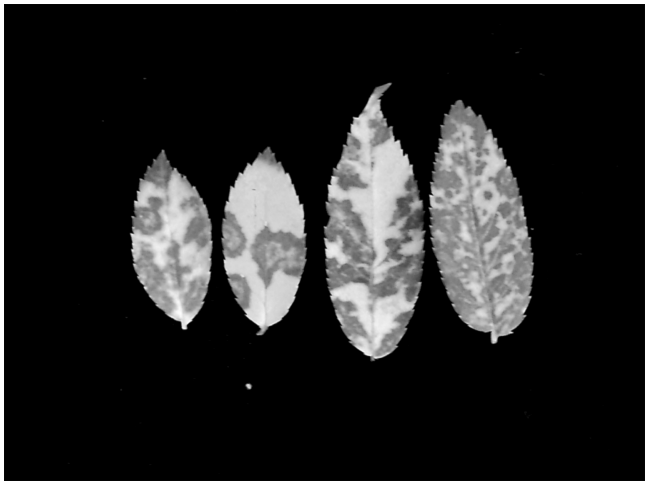

Fig. 6.2. Disease symptoms on leaves of *Rosa* 'Ballerina' infected with the newly described filamentous virus RoYMV. (*Source*: Courtesy of Ben Lockhart, University of Minnesota.)

III. RESISTANCE SCREENING

A. Black Spot

For purposes of genotype evaluation and breeding, resistance to rose black spot disease can be broadly categorized into race-specific resistance and partial resistance types. The aforementioned inoculation tests that have differentiated *D. rosae* pathogenic races in North America and in Europe have done so on the basis of clearly discerned compatible and incompatible interactions. For example, Debener et al. (1998) rated interactions susceptible (compatible) if the fungus grew outside the original spore suspension droplet and if spore-bearing acervuli were formed on the leaflet surface. Resistant (incompatible) reactions, however, were characterized by exhibiting neither of these symptoms. Such resistance can be conferred by rapid host cell death called the hypersensitive response (HR), which was documented in 'Allgold' rose by Kuklinski (1980) and in *R. wichuriana* and *R. roxburghii* by Wiggers et al. (1997). Blechert and Debener (2005) observed two types of HR in species roses, one in which single cells died and one in which larger cell clusters died. The latter response was presumably due to a delay in HR until after some fungal development within the host tissue had already occurred. They also reported another type of resistance in which no fungal structures penetrated below the

leaf cuticle. Similarly, Reddy et al. (1992) observed the death of spores on the leaf surfaces of resistant species and concluded that spore development halted at germination on these species, possibly due to germination inhibitors exuded from the leaf epidermis. This result is consistent with reduced conidial germination on the leaflet surface of the resistant 'Allgold' (Knight and Wheeler 1978b).

Partial resistance is an incomplete form of resistance characterized by suppression of pathogen colonization after infection. This type of resistance is generally considered to be race nonspecific. Blechert and Debener (2005) described multiple interaction types on 34 different rose species, separating susceptible reactions into five different classes based on hyphal morphology, location, and length. In some susceptible reactions acervuli were produced, but subcuticular and short-distance hyphal growth was restricted. Kuklinski (1980) described black spot infections in which haustoria formed but were retarded in development or became enclosed in a ''convoluted'' membrane. It appears that the cuticle can also play a role in partial resistance, and it may be advisable in some cases to abrade the leaflet surface prior to inoculation in order to eliminate this variable (Castledine et al. 1981). On a macroscopic level, partial resistance reactions can be observed according to the timing of development as well as size and number of black spot lesions. Xue and Davidson (1998) formalized these observations into five measures, or ''components,'' of partial resistance that were used to screen roses from the Agriculture and Agri-Food Canada breeding program in Morden, Manitoba. These are incubation period (IP), leaf area with symptoms (LAS), number of lesions (NL), lesion length (LL), and sporulation capacity (SC). Four of the components, LAS, IP, NL, and LL, were positively correlated with one another, although SC was not significantly correlated with IP, NL, and LL. From this analysis, the authors concluded that, in light of limited resources, LAS and SC were the two most useful components, although SC is more difficult to measure than the visual rating of LAS (Fig. 6.3).

Leaf drop may be considered another component of partial resistance. It is not well correlated with lesion development (Kuklinski 1980; Whitaker et al. 2007c), since some cultivars may retain their leaves despite severe infection, while other mildly infected clones drop their leaves rapidly. In one example, rooted cuttings of a diploid rose accession (H71) dropped all their leaves only 12 hours after inoculation and subsequently grew disease-free shoots (Svejda and Bolton 1980). Such a mechanism may effectively sanitize the plant by rapid removal of future inoculum sources. The production of ethylene by *D. rosae* combined with auxin degradation may contribute to leaf

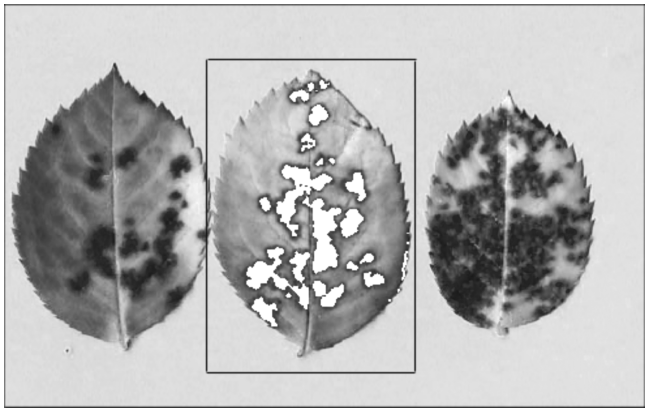

Fig. 6.3. Rose leaflets infected with *D. rosae*. The partial resistance rating for the center leaflet (lesions highlighted) was 20.3% leaf area with symptoms (LAS), as measured with Assess Software. (*Source*: APS Press, St. Paul, MN.)

yellowing and abscission (Horst 1983; Kazmaier 1961). Ethylene has been implicated in the premature defoliation that occurs as a result of several diseases and is known to be produced by fungal and bacterial pathogens (Agrios 1997). If leaf abscission in response to black spot infection is correlated with general ethylene sensitivity, ethylene applications may be used to screen against early leaf drop.

Single-spore isolates are important for studies of both race-specific and partial resistance. Without the ability to isolate and produce inoculum from single spores, inoculation results may be inconsistent, depending on what combination of races is present when spores are collected in the field. Palmer and Seminuk (1961) collected spores from the field because of their immediate need for large amounts of inoculum. However, interpretations of their results were limited because the races they inoculated were unknown. Producing inoculum from single spores in culture may be worth the wait, since large numbers of spores can be produced from cultures on PDA in 7 to 8 weeks, with tens of millions of spores produced from each plate (Xue and Davidson 1998). If fewer inocula are required, single-spore isolates produced in culture may be inoculated to surface-sterilized leaves. The infected leaves may be frozen and subsequently thawed to harvest spores.

Detached leaf assays and leaf disc assays with single-spore isolates have been used successfully for determining races and identifying the presence and absence of major resistance genes (Debener et al. 1998; Von

Malek and Debener 1998; Yokoya et al. 2000). Whole-plant inoculations have been used so far in studies of partial resistance (Xue and Davidson 1998; Whitaker et al. 2007c). Since detached leaf assays are efficient and can be performed in the lab, they would also be convenient for screening partial resistance. Jenkins (1955) reported a near-perfect correlation coefficient ($r = 0.97$) between a comparison of lesion sizes in detached leaflet and whole-plant assays for four cultivars and one species inoculated with three isolates of *D. rosae*. This suggests that finer distinctions between levels of susceptible reactions also may be measured using detached leaves. Other research, however, raises the question of whether detached leaf assays are accurate for measuring partial resistances. Walker et al. (1996) documented susceptibility on detached leaflets of 'Alberic Barbier' rose, which was noted to be resistant in the field. In addition, the growth of *D. rosae* was repressed by cell suspensions from 'Alberic Barbier' root callus. From this it was suggested that diffusible substances produced away from leaves could have a role in resistance. Palmer et al. (1966a) reported that the deterioration of detached leaflets made rating impractical for some rose genotypes. They also noted that this method may not accurately measure the effects of vigor in whole plants. Knight and Wheeler (1978a) commented on a lack of whole plant realism in leaf disc assays, but suggested that leaf disc assays could serve as a complement, rather than a replacement, to field trials. It also should be noted that the defoliation response of the plant to infection is important and can be determined only using whole plants. Therefore, the correlation between whole plant and detached leaflet methods should be tested before the latter is utilized for genetic studies of partial resistance against multiple races. Such experiments are currently under way at the University of Minnesota (unpublished data).

Black spot screening in commercial breeding programs still is carried out in the field (Noack 2003). Yet Carlson-Nilsson (2002) compared controlled inoculation data and field data and concluded that greenhouse inoculations of whole plants could be used as a substitute for field trials. Greenhouse inoculations have advantages over natural field inoculations, since it takes several years for pathogen pressure to build up in field plots (Carlson-Nilsson 2000). Also, correlations between disease ratings among years can be low due to nonuniform spread of inoculum and climatic differences (Leus 2005). Greenhouse screening is only effective when high humidity is maintained for at least 24 hours after inoculation. This can be accomplished using plastic tents. Leus (2005) found that for seedling selection in the greenhouse, it is best to

apply two or three repeated inoculations. They also found that an inoculum concentration of only 2×10^2 conidia/ml was sufficient when inoculations were repeated.

Although race-specific resistance and partial resistance are useful categories in terms of breeding and genetics, they do not directly describe the underlying biology of plant defense. Plant response to pathogen attack involves a myriad of pathogen receptors, signal transduction pathways, and defense-related gene products. Some defenses are constitutive, but other defense compounds are induced only after pathogen attack. For example, polyphenol and phytoalexin production increase during black spot infection (Saunders 1967). Two categories of inducible resistance are called systemic acquired resistance (SAR) and induced systemic resistance (ISR). They are differentiated by the nature of their signaling cascades. SAR is dependent on salicylic acid as a signal molecule, and ISR is dependent on jasmonic acid and ethylene signaling (Van Loon 1999). Several families of pathogenesis-related (PR) proteins have been identified that are actively involved in resistance in many species. Interestingly, PR proteins are associated with SAR but not with ISR (Van Loon 1999). Several families of PR proteins (PR-1, PR-2, PR-3, and PR-5) accumulate in *D. rosae*–infected rose leaves, and all except PR-1 are systemically induced in uninfected upper leaves (Suo and Leung 2002a). It also appears that the expression of two specific types of PR proteins, β-1,3-glucanase and chitinase, contribute to the reduction of black spot symptoms on in vitro rose plants. These antifungal compounds are known to degrade fungal cell walls into short oligosaccharides, which may serve as signals for other downstream defense responses (Suo and Leung 2001a).

Screening for differences in induced resistance may be possible by inducing SAR with synthetic compounds such as acibenzolar-S-methyl (BTH), which is thought to affect the SAR signal transduction pathway downstream of salicylic acid (Ryals et al. 1996). Pathogenesis related proteins can be induced within 3 days of BTH treatment, and BTH treatment prior to inoculation of in vitro plantlets reduces black spot symptoms in rose (Suo and Leung 2001b, 2002b). Systemic acquired resistance may be a mechanism underlying the partial resistance phenotype in rose. If so, BTH applications to selected genotypes would distinguish their potential for partial resistance.

B. Powdery Mildew

In 1966, Mence and Hildebrandt tested the resistance of various rose cultivars and species using polysporous inoculum on detached leaves.

Their results allowed several initial insights into the resistance of roses to *P. pannosa* and implications for screening methods. Inoculations were performed by gently brushing clean leaflets with infected leaflets. Although they did not utilize single-spore isolates, host-specificity was nonetheless apparent, as isolates collected from *R. virginiana* were not infective on some cultivars. Degree of resistance was rated for susceptible genotypes according to the percentage of leaf area covered by sporulating colonies. It was noted that susceptibility decreased dramatically with leaf age. Some roses may become resistant more quickly than others as their leaves age. For instance, 'Queen Elizabeth' became resistant at a faster rate that 'Christopher Stone' (Mence and Hildebrandt 1966). Therefore, in order to avoid the genotype-specific effects of aging, leaves must be inoculated within a few days of unfolding. The cuticle was deemed to have little role in resistance since abrasion had a negligible effect on susceptibility (see also Conti et al. 1985), although conflicting evidence on this point has been presented for other genotypes (Ferrero et al. 2001b). *Rosa rugosa* leaflets were susceptible only when inoculated on the abaxial surface; all other genotypes were effectively inoculated on both surfaces.

Since this initial study, resistance has been identified in other commercial cultivars as well as in species such as *R. multiflora, R. wichuriana, R. laevigata* × *R. rugosa* hybrids, and others from sections *Caninae* (*R. agrestis* and *R. glutinosa*) and *Pimpinellifoliae* (*R. foetida* var. *persiana*) (Ferrero et al. 2001a; Linde and Debener 2003; Linde and Shishkoff 2003). Chinese accessions of *R. sterilis* and *R. chinensis* are reported as resistant, with different accessions of *R. roxburghii* exhibiting a wide range of symptoms (Wen et al. 2006). Screening methods have been improved over the years, and single-spore isolates are now utilized for the differentiation of races and screening of populations for resistance genes (Bender and Coyier 1984; Linde and Debener 2003; Leus et al. 2006).

Unlike black spot disease, germination of *P. pannosa* conidia on the leaf surface does not appear to be related to host resistance and is not a good indicator of susceptibility (Conti et al. 1985). Instead, resistance mechanisms are initiated only during or after penetration. Host cell death is a common means of resistance and appears to be associated with the accumulation of phenolic compounds (Conti et al. 1986) and hydrogen peroxide (Dewitte et al. 2007). The HR is localized to single cells in some resistant genotypes, but a delayed HR may affect multiple cells and be visible to the naked eye (Linde and Shishkoff 2003). Although necrosis of groups of cells can be indicative of resistant genotypes (Wen et al. 2006), browning of multiple cells also can occur

in conjunction with sporulation and mycelial development (Dewitte et al. 2007). Therefore, the occurrence of visible browning is not by itself a sufficient indicator of resistance. In other cases, resistance mechanisms of roses such as *R. laevigata anemoides* may include papillae formation and abnormal haustoria in the absence of any cell reaction or collapse (Dewitte et al. 2007). Therefore, most rating systems measure the degree of sporulation on infected leaves, since this is the most reliable and most easily observed indicator of susceptibility.

If disease measures such as percent of leaf area covered with conidiophores are to be used, care should be taken to quantify spore density during inoculations and apply inoculum evenly. This is not possible when using infected leaves or camel's-hair brushes to apply *P. pannosa* conidia. Therefore, settling towers that use compressed air to disperse dry conidia have been developed. Linde and Debener (2003) used such an inoculation tower to distribute approximately 2 conidial/mm^2 onto detached leaflets. Spores were captured and measured by placing microscope slides alongside the leaflets. Leus (2005) encountered high variability and low reproducibility of disease scores using an inoculation tower and recommended several replicate inoculations when using this method. A disadvantage of this strategy is that inoculum density cannot be fixed beforehand. This is because spores are released into the tower by using compressed air to blow them off infected leaves. In addition, only a limited number of genotypes may be inoculated simultaneously.

Maintaining high humidity around inoculated leaflets is advisable for promoting optimum infection. Incubating inoculated leaflets at 95% relative humidity (RH) promotes significantly more sporulation than incubating at 50% RH (Hural and Coyier 1985). However, free water has a negative effect on infection if present during the first 6 hours after inoculation. Although germination of conidia is not affected, the penetration of the germ tube is inhibited by free water (Perera and Wheeler 1975).

Yan et al. (2006) were able to develop a liquid spore suspension inoculation method that avoids the negative effects of free water on infection. This was accomplished by spraying young cuttings with a concentration of 10^3 to 10^4 conidia/ml and immediately increasing the temperature of a climate controlled compartment to 28°C for 15 minutes to promote evaporation. They then lowered the temperature to 22°C for the duration of the experiment. This method is advantageous for determination of partial resistance because it allows the uniform application of a predetermined concentration of spores on a large number of plants at the same time. The partial resistance

components rated were the percentage of leaf area covered with symptoms, latent period (LP), and rate of symptom development (RSD). The RSD was calculated as the ratio of the percent leaf area at 11 days postinoculation to the time interval from the first appearance of lesions until 11 days postinoculation.

As with black spot, one could ask whether detached leaf assays are suitable for screening partial resistance to powdery mildew. The data for answering this question conclusively are not yet available. In one instance, lines containing an antimicrobial transgene differed in their disease scores when inoculated as detached leaves versus whole plants (Li et al. 2003). Of seven lines tested, three differed widely in their disease scores between the two assays. However, different inoculation methods were used for the two assays, and spore concentrations were not quantified in the whole-plant assay.

Leus et al. (2003) developed a greenhouse screening protocol in which susceptible plants of 'Pfander's Canina' were planted intermittently among seedlings. Conidia from these plants were dusted onto the seedlings. Increased infection in this greenhouse experiment resulted in much higher selection intensity based on powdery mildew resistance compared with greenhouses not containing the 'Pfander's Canina' plants (Leus 2005). Disease ratings from both greenhouse treatments were well correlated with field infections. From this study, the authors recommend an initial screening of young seedlings in the greenhouse followed by screening of advanced selections with the most virulent pathotypes (Leus et al. 2003).

As with BTH, treatment with 2,6-dichloroisonicotinic acid (INA) also can be used to chemically induce SAR. This approach was taken by Higwegen et al. (1996) to compare the powedery mildew resistance of two roses, 'Madelon' and 'Sonia'. Conidia were dusted on plants 4 days after INA application, and ratings were performed 18 days postinoculation. Both cultivars exhibited decreased colony formation and spore production in response to INA as well as early necrosis, haustorial encasement, and polyphenol production. Interestingly, 'Sonia' had increased partial resistance after INA treatment compared to 'Maledon', exhibiting slightly less colony formation and a marked decrease in sporulation capacity. Leus (2005) examined the effectiveness of SAR against powdery mildew by applying BTH and Milsana® (an extract of giant knotweed, *Reynoutria sachalinensis*) to cuttings of 'Excelsa'. Both treatments significantly decreased infection. Leus (2005) also demonstrated the presence of ISR in roses against powdery mildew, which can be induced by nonpathogenic soil bacteria, specifically *Pseudomonas* strains. These strains, when applied to the roots of rose cuttings prior to

inoculation, caused a decrease in disease symptoms similar to BTH. These methods make it possible to evaluate both SAR and ISR in roses and could be used to screen for non–race-specific resistance against powdery mildew.

C. Other Diseases

Botrytis cinerea screening can be accomplished by spray inoculation methods with conidia that are produced in culture. Evidently, no complete resistance to this fungus has been found, but small differences in resistance have been found among cultivars that are indicative of partial resistance. Pie and Brouwer (1993) observed that some cultivars had reduced disease severity that was due to decreased hyphal growth after germ tube penetration. Unfortunately, symptoms still were visually evident, and it was suggested that a form of resistance that prevented penetration would be more effective. Hammer and Evensen (1994) attributed the resistance of one cultivar to a decrease in germ tube penetration. However, the difference in disease severity among genotypes was small and noticeable only at high inoculum concentrations. Discovery of more effective resistances against *B. cinerea* would be valuable.

Cercospora leaf spot usually is considered a minor fungal disease of roses, but infestations can cause significant spotting and defoliation of roses under hot, humid conditions in the United States (Hagan et al. 2005). In a 5-year field study of the resistance of rose cultivars to fungal diseases in Alabama, few cultivars were susceptible to both black spot and leaf spot. Interestingly, all cultivars with high resistance to black spot were infected with Cercospora leaf spot. Among those cultivars susceptible to leaf spot, significant differences in resistance were observed that were consistent over all years (Hagan et al. 2005). These differences may provide a genetic basis for breeding. The results also raise questions about the extent of correlation between resistance to some pathogens and susceptibility to others.

Downy mildew may be artificially inoculated by spraying conidia of *Peronospora sparsa* on leaf surfaces. Maximum infection can be obtained when temperature is maintained at or below 18°C and leaf wetness is maintained for 120 hours (Xu and Pettitt 2003). Schulz and Debener (2007) artificially inoculated detached leaves and rated downy mildew symptoms using a 5-step scale based on conidiophore production on the lower leaf surface. They reported that 13 of 84 wild species tested were resistant based on this scale, 6 of which are from section *Caninae*. Although oogonia are formed primarily within

necrotic lesions, conidiophores usually are present at the lesion margins and even within the green, apparently uninfected portions of the leaf (Xu and Pettitt 2003). Therefore, when screening for resistance, extralesion formation of conidia should be considered. Detection of conidia or other pathogenic structures prior to visible formation of symptoms is possible with a PCR-based assay specific to the pathogen's internal transcribed spacer (ITS) region (Aegerter et al. 2002). A protein-based approach using enzyme-linked immunosorbent assay (ELISA) is also effective for early detection. ELISA results correlated well with the visual estimations of conidiophore production in the field (Schulz and Debener 2007). Because initial downy mildew symptoms may be easily confused with those of black spot or powdery mildew, methods for early diagnosis are quite useful.

Although rose rust species (*Phragmidium* spp.) are usually specific to certain roses, some rose hosts may be infected by multiple rusts. For example, *P. tuberculatum* and *P. mucronatum* could not be distinguished on some rose species in a European survey using morphological characters, and it was suggested that molecular methods of identification would be more successful (Leus and Van Huylenbroeck 2007). Importantly, field ratings of rust infection revealed marked differences between rose species that may serve as a basis for resistance breeding efforts. For instance, *R. rubiginosa* had a low disease index whereas *R. canina* was severely infected.

Spot anthracnose symptoms can be rated with methods similar to methods used for black spot, but care must be taken not to confuse young spot anthracnose lesions with black spot lesions. Uggla and Carlson-Nilsson (2005) used a simple percentage rating scale to assess spot anthracnose in field plots over 2 years. Families from interspecific crosses within section *Caninae* were compared. Families with *R. rubiginosa* as the female parent differed significantly depending on the male parent. These differences offer a sound basis for selection among families and the first evidence for variation in combining ability for resistance to spot anthracnose.

Inoculations of *A. tumefaciens* can be performed by pricking rose stems with a bacteria-laden needle or applying bacterial solution to vertical stem cuts. After 6 to 8 weeks, crown galls can be rated by measuring percent incidence and gall diameter (Aloisi et al. 1998; Zhou et al. 2001). Aloisi et al. (1998) established that there are significant differences in resistance among rose genotypes. Genotypes of *R. indica* and *R. multiflora* exhibited a large range of symptoms, ranging from 100% of plants affected for some genotypes to only 4% of plants affected for others. A separate test utilizing both in vitro and

in vivo inoculation techniques indicated a range of susceptibility for *R. multiflora* genotypes and also confirmed several cultivars including 'Double Delight' and 'Fire' as highly resistant (Zhao et al. 2005). Suo and Leung (2001b) reported differences among cultivars, with 'Madam Isaac Pieriere' exhibiting 70% gall formation as compared to 25% for 'Alexander'. Screening for induced resistance to crown gall also may be possible using chemical compounds. Crown gall incidence of in vitro inoculated plantlets of 'Madam Isaac Pieriere' was reduced by 26% after pretreatment with BTH (Suo and Leung, 2001b). Whether the acquired resistance was systemic or merely local was not clear.

Due to the belowground location of nematode infection, inoculation of plants with nematodes is more difficult than for foliar pathogens. Nevertheless, French researchers have successfully inoculated rose rootstocks of various rose species with the root-knot nematode *Meloidogyne hapla*, demonstrating a wide range of genetic resistance (Voisin et al. 1996). Tomato plants with galled roots were grown in the same pot with the rose genotypes for 2 months until the top part of the tomato plant was removed. After 90 days, symptoms were assessed. Based on root gall ratings and counts of nematodes extracted from roots, *R. manetti* genotypes were the most resistant, followed by *R. canina. Rosa multiflora* genotypes were variable in their resistance.

IV. BREEDING

A. Resistance Genes

The genetic nature of disease resistance is important in rose breeding. Resistance may be conferred by single genes or by many. Alleles may be dominant or recessive. Certain genes may confer race-specific resistance, while others may be non–race specific. These and other aspects of host genetics and biology determine which breeding strategies will be most effective.

The first black spot resistance gene to be described is *Rdr1*, which is a major, race-specific resistance gene (*R* gene) (Von Malek and Debener 1998). Genetic studies were carried out with a tetraploid line developed from a resistant *R. multiflora* that was chromosome doubled and crossed to the tetraploid 'Caramba'. Segregation ratios of resistant to susceptible progeny from selfing (35:1), backcross to the susceptible parent (5:1), and crosses to three different susceptible cultivars (5:1) were consistent with a single, dominant gene in duplex (*RRrr*) configuration. Discovery of this *R* gene was the first direct evidence

of a gene-for-gene interaction in any rose pathosystem. The region around this gene was fine-mapped using AFLP markers, and bacterial artificial chromosomes (BACs) spanning this locus were identified using genomic DNA from *R. rugosa* (Kaufmann et al. 2003). Another gene, *Rdr2*, was discovered that is effective against a different isolate of black spot than *Rdr1*. By examining its segregation in two diploid populations, it was determined that it is tightly linked to *Rdr1* (Hattendorf et al. 2004). *Rdr1* resides within a cluster of 8 sequences, each containing a nucleotide binding site and a leucine-rich repeat. Because it lies in such a cluster, identification of the actual gene may require separate transformation of susceptible roses with each candidate sequence (Debener 2005).

Another resistance gene in roses is *Rpp1*, which confers race-specific resistance to powdery mildew and gives evidence of gene-for-gene interactions in the rose–powdery mildew pathosystem (Linde and Debener 2003). In a backcross to the susceptible parent, a 1:1 segregation ratio indicated that this trait is conferred by a single dominant gene. Transgressive segregation occurred in which the disease index of some susceptible progeny far exceeded that of the susceptible parent, possibly due to minor alleles with negative effects. Considerable transgressive segregation for powdery mildew resistance was also observed by Yan et al. (2006), indicating heterozygosity for resistance genes in the parents.

Major *R* genes typically confer complete resistance but only against a limited number of fungal races. These resistances can be compromised quickly through pathogen mutation or migration, which has occurred for rose black spot (Bolton and Svejda 1979; Yokoya et al. 2000). Yet single dominant genes have the advantage of being easy to select for using detached leaf assays and can be selected for early in the breeding process. They also may be combined together in single genotypes in a pyramiding scheme to produce broader resistance. To date, a gene-pyramiding approach for disease resistance has not been demonstrated in rose.

Partial resistance to disease in plants generally is assumed to be controlled by many genes and to be non–race specific (Simmonds 1991). Therefore, searching for quantitative trait loci (QTL) is a logical fist step in the genetic analysis of partial resistance. Two QTL for powdery mildew resistance were identified in a population of diploid F_1 plants arising from a cross between a cultivated diploid rose and *R. wichuriana* (Dugo et al. 2005). Disease symptoms were scored on a simple 0 to 2 scale over one field season, and the two QTL *Pm1* and *Pm2* accounted for 45.2% and 24.9% of phenotypic variability

respectively. In a larger study that included multiple disease scores, inoculation methods, and both field and greenhouse assessment, QTL were analyzed in a diploid population (Linde et al. 2006). In order to focus on the genotypes with the most extreme disease ratings, a selective genotyping scheme was employed in which 170 of the 270 phenotyped plants were included in the analysis. This method was effective, resulting in the discovery of 28 QTL contributing to powdery mildew resistance, while saving time and labor necessary to genotype the entire population.

Partial resistance to powdery mildew has utility in breeding as demonstrated on a progeny mean basis (Leus 2005). Although one progeny had a mean disease rating exceeding 80%, another consistently exhibited less than 30% infection. These results were consistent in both the field and greenhouse. This approach is a practical way to gain information about the combining ability of the parents and their potential to transmit partial resistance in other crosses. No QTL for black spot resistance have been described, but continuous distributions of traits among some progenies are indicative of polygenic control (Carlson-Nilsson 2000; Uggla and Carlson-Nilsson 2005).

Resistance to black spot and powdery mildew in roses has a clear quantitative aspect, but one should not assume that partial resistance in roses is always quantitative and non–race specific. Partial resistance to wheat leaf rust (Rubiales and Niks 1995) and leaf blast of rice (Zenbayashi et al. 2002) is conferred by single genes. Race-specific partial resistance has been documented in several pathosystems (Parlevliet 2002), including the discovery of isolate-specific QTLs for barley leaf rust (Qi et al. 1999), clubroot of *Brassica sp.* (Rocherieux et al. 2004), and apple scab (Durel et al. 2003). QTL studies with multiple races of rose pathogens could be conducted to determine if these exist for rose powdery mildew or black spot. Some evidence already exists, as Linde et al. (2006) reported a QTL for powdery mildew race 9 that was not detected in the greenhouse and field tests with polysporous inoculum. In addition, some QTLs for powdery mildew occurred in regions that contained resistance gene analogs (RGAs), although most QTLs did not colocalize with RGA clusters. This concept is further supported by an inoculation of two single-spore isolates of powdery mildew to a tetraploid rose population arising from a cross between two partially resistant parents (Yan et al. 2006). The isolates differed in their overall pathogenicity on this population. Moreover, the population exhibited race specificity for partial resistance, resulting in a highly significant genotype × isolate interaction. Similarly, in an inoculation of 12 rose genotypes with 14

single-spore isolates of black spot, Whitaker et al. (2007c) demonstrated that partial resistance for some rose genotypes varied with isolate. Recently multiple inoculations confirmed the race dependence of lesions size for at least one rose genotype (unpublished data). Genetic studies are currently under way to determine the inheritance of this trait.

Genetic analyses of resistance have been conducted for spot anthracnose, crown gall disease, and nematode infection. Resistance to spot anthracnose in a single study appeared to be quantitative, which is indicated by family mean differences for progenies with different male parents (Uggla and Carlson-Nilsson 2005). However, these results must be interpreted with caution, as multiple pathotypes may have been present in the field. The genetic basis of resistance to crown gall was studied in self and F_1 progenies of the resistant 'PEKcougel' and the susceptible 'Dukat' (Zhou et al. 2001). Disease incidence in the 'PEKcougel' self-pollinated progeny was significantly lower than for the other progenies, and a continuous distribution of resistance in all progenies indicated multigenic control. Similarly, resistance to the nematode *M. hapla* in *R. multiflora* and *R. indica* is controlled by several minor genes, which was inferred from monomodal distributions of progenies from a partial diallel crossing scheme (Wang et al. 2004). Although race-specificity of resistance was discovered in *R. indica*, crosses that would have allowed analysis of this resistance were not reported.

B. Breeding Methods

About 130 species of roses are cataloged worldwide (Zlesak 2006). Yet most of the recurrent-flowering or reblooming rose cultivars in our gardens are tetrasomic tetraploids ($2n = 4x = 28$) originating from 10 to 15 mostly diploid ($2n = 2x = 14$) species (De Vries and Dubois 2001). Various fertility barriers, reviewed by Gudin (2000) and Zlesak (2006), limit the parental germplasm base and restrict the size of progeny populations in this highly heterozygous, outbreeding species complex. Since cultivated germplasm is derived from such a small proportion of known species, introgression of disease resistance traits from species roses has potential. However, this usually involves transmission of desirable traits from the diploid to the tetraploid level.

The transfer of disease resistance traits across ploidy levels may be accomplished by several methods. Colchicine doubling of wild species followed by crossing with cultivated tetraploids is the most-used method (Von Malek and Debener 1998), although decreased fertility in

the original 4*x* induced plant can occur (Zlesak et al. 2005). Furthermore, inbreeding depression of hybrids may result after successive generations of backcrossing. To avoid this result, different cultivated varieties can be used for each backcrossing cycle rather than using a single clone as the recurrent parent (Debener 2000). A slightly different approach is to cross two wild rose species and then chromosome double the resultant seedlings, which are called amphidiploids. Basye (1990) pioneered this work by doubling *R. banksiae* × *R. laevigata* hybrids with colchicine. The same methods were later used to create amphidiploids from other species (Ma et al. 1997). It was determined that these amphidiploids are more fertile than the original diploid hybrids, although they still have only ''low to moderate'' fertility. Some have been successfully hybridized with commercial varieties to transmit resistance into cultivated forms (Byrne et al. 1996).

Polyploidization with trifluralin is also effective, as demonstrated in *Rosa chinensis minima*, and promises to be a cheaper and safer alternative to colchicine (Zlesak 2005). Oryzalin is also a desirable compound for spindle inhibition since it is poses less carcinogenic hazard to animal cells. It may not induce chromosomal abnormalities and DNA mutations in plants, as does colchicine (Kermani et al. 2003). Oryzalin may be used for chromosome doubling of shoot meristems and nodal sections of desirable genotypes, as demonstrated by Kermani et al. (2003). This method allows the doubling of chromosomes in clones as opposed to seedlings. Tetraploid plants were recovered at a rate of 66% from a 1-day treatment of nodal sections with 5 μM oryzalin. Importantly, chromosome doubling significantly increased pollen viability for the tetraploid forms of four diploids and for the hexaploid form of a triploid hybrid. Doubling of diploids and triploids with low fertility, therefore, has potential for introgression of resistance. However, one intriguing instance provides reason for caution. A diploid hybrid from a cross of 'Martin Frobisher' × 'Mistress Quickly', resistant to three races of *D. rosae*, became susceptible to all three races after polyploidization to tetraploid forms (Allum and Roberts 2005; A.V. Roberts, pers. comm.). Chromosomal loss and/or mutation could be the cause of this phenomenon and would be difficult to rule out. Other possible explanations are epigenetic changes resulting from polyploidization that could alter expression of *R* genes or gene dosage effects. Experiments to further elucidate the effect of ploidy on susceptibility are currently being conducted.

An alternative to chemical induction of polyploids is sexual polyploidization, which has the potential to preserve fertility and

prevent inbreeding depression by maintaining heterozygosity. One way to increase ploidy level by sexual means is through exploitation of unreduced gametes. Crespel et al. (2002a) demonstrated the presence of both first-division restitution (FDR) and second-division restitution (SDR) mechanisms in rose dihaploids that produced fertile 2*n* gametes. Three segregating populations that resulted from crosses of the dihaploids with rose species yielded an average of 66% polyploidy progeny. AFLP analysis of the parents and progeny revealed that some FDR events occurred with no meiotic crossover (FDR-NCO), contributing 100% of parental heterozygosity to the progeny. Polyploids may be produced by crossing two different 2*n* gamete-producing genotypes with each other or by crossing normal diploid or polyploid roses with 2*n* gamete-producing genotypes (El Mokadem et al. 2002). Detailed information on the rate of incidence, heritability, and genetic transmission of unreduced gamete production is not yet available and would be of considerable value to rose breeders.

A second sexual polyploidization approach involves creating a ploidy bridge by crossing wild rose species with tetraploids to generate triploids, which are subsequently crossed with cultivated tetraploid germplasm to produce tetraploids. Triploid roses are generally considered to be sterile, but Leus (2005) showed that, although pollen viability of triploids is low compared to tetraploids, a small percentage of pollen (0–10%) is still functional. Crosses between male triploids and female tetraploids primarily produced tetraploid offspring (98%), suggesting a competitive advantage for diploid pollen over monoploid pollen. By utilizing AFLPs and microsatellite markers, Leus (2005) demonstrated that DNA from diploid parents was transmitted to tetraploid progeny by this method.

When selecting species for use in the introgression of disease resistance genes, it is important to note that members of the same species may differ widely in their resistance. Palmer et al. (1966a) reported differences in black spot resistance between two different clones of *R. multiflora* and between two clones of *R. wichuriana*. Similarly, two different clones of *R. wichuriana* differed widely in an inoculation with German isolates, with one clone resistant to all five races and the other susceptible to all races (Debener et al. 1998). Until the 1970s, *R. rugosa* was considered to be resistant to black spot, but 'Martin Frobisher' was suddenly overcome by a new race in Ottawa (Bolton and Svejda 1979), with susceptibility of *R. rugosa* later demonstrated in Europe as well (Debener et al. 1998). Although *R. spinosissima* is thought to be quite disease resistant, one clone was found to be susceptible to three North American races of black spot,

with up to 48% of the leaf area affected (Whitaker et al. 2007c). For powdery mildew, two clones of *R. multiflora* exhibited different race specificities when inoculated with *P. pannosa* (Linde and Debener 2003). For crown gall, *R. indica* and *R. multiflora* clones varied widely in their resistance to *A. tumefaciens* biovar 1 (Aloisi et al. 1998). And finally, Voisin et al. (1996) found marked differences among *R. multiflora* species for resistance to the nematode *M. hapla*. Therefore, species should be screened with multiple pathogenic isolates prior to selecting clones for breeding purposes.

A significant drawback to introgression of disease resistance traits from wild genotypes is the time frame involved. Ten or more years are needed to develop cultivars starting from species roses, as several generations of crosses are required to eliminate the wild background. A potentially quicker approach would be the detection and utilization of disease resistance traits already present in cultivated germplasm. Another advantage of this approach is that polyploidization can be avoided altogether by utilizing resistant varieties. Inoculations designed to differentiate pathogenic races of black spot (Svejda and Bolton 1980; Spencer and Wood 1992a,b; Debener et al. 1998; Yokoya et al. 2000; Leus 2005; Whitaker et al. 2007c) and powdery mildew (Linde and Debener 2003) have identified race-specific resistances in commercially available cultivars. Although a number of modern cultivars are triploid, many are fertile tetraploids as well (Leus 2005). The utility of such resistances will depend on whether they can be pyramided together effectively to combat multiple pathogenic races. Roses included in these inoculation tests that are wild species or selections that are closely related to wild species tend to have broader resistance in that they are usually resistant to more fungal races than the cultivars. Ultimately, the approach taken must depend on the goal of the breeding effort. If the breeder aims to introduce hardy, landscape-type roses, wild species can be used with less extensive backcrossing (Debener 2000). The gene pool of florist roses is much narrower, and a scheme that utilizes resistances from cultivated germplasm would be most practical.

Disease resistance traits do not exist in a vacuum. Flower color and form, vigor, scent, stem quality, and other traits are vital in commercial breeding programs. Variable offspring originating from highly heterozygous parents means that large progenies are required in order to obtain the optimal combination of traits. In a typical commercial breeding scheme presented by Noack (2003), at least 100,000 seedlings are grown yearly, of which about 3% are selected after the first year for replicated testing. Since most repeat blooming roses flower as young seedlings, there is heavy early selection for floral

characters. More resources and time are required to assess disease-resistance characteristics, so gain from selection for disease resistance may be very slow (Zlesak 2006). Since early rouging of seedlings conserves valuable resources, marker-assisted selection (MAS) could be a way to conduct early selection for resistance, concurrent with selection for floral traits. This would require that markers linked to disease-resistance traits are available and could be applied in a cost-effective manner (Noack 2003). Early screening of black spot and powdery mildew resistance can be performed by inoculating in a greenhouse. On the surface, inoculation appears to be a lower-cost approach compared to MAS. However, the presence of race specificity for major genes and QTLs means that multiple inoculations with different races should be performed. This consideration might lower the relative cost of MAS. In addition, resistance conferred by major genes can obscure the presence of partial resistance QTLs, which can be detected using molecular markers.

V. MOLECULAR TOOLS

A. Markers and Mapping

Discovery of DNA markers closely linked to resistance genes and QTLs may facilitate MAS and is usually a prerequisite for gene discovery and cloning. Comparing different genetic maps within and outside of plant species also can speed up the discovery of new markers and resistance gene candidates. Mapping of disease-resistance genes in roses began with the discovery of markers linked to *Rdr1* via bulk segregant analysis (Michelmore et al. 1991) of a segregating tetraploid population (Von Malek et al. 2000). Seven AFLP markers linked to the gene were identified, one of which was converted to a sequenced characterized amplified region (SCAR) marker and localized on a diploid map of rose (Fig. 6.4). This diploid map was constructed previously using 60 F_1 plants in a double-pseudotest cross design and was the first linkage map of rose (Debener and Mattiesch 1999). Bulk segregant analysis with AFLP also was used to find markers linked to powdery mildew resistance gene *Rpp1* (Linde et al. 2004). An AFLP marker closely linked to *Rpp1* was converted into SCAR marker, which was found to be unlinked to the *Rdr1* locus in a diploid population segregating for both genes. Although *Rpp1* was localized on linkage group 3, *Rdr1* was localized to linkage group 1. The powdery mildew QTLs of Linde et al. (2006) were mostly clustered on linkage groups 3 and 4. The cluster colocalizing with a locus for the presence/absence of prickles on linkage group 3 accounted for around

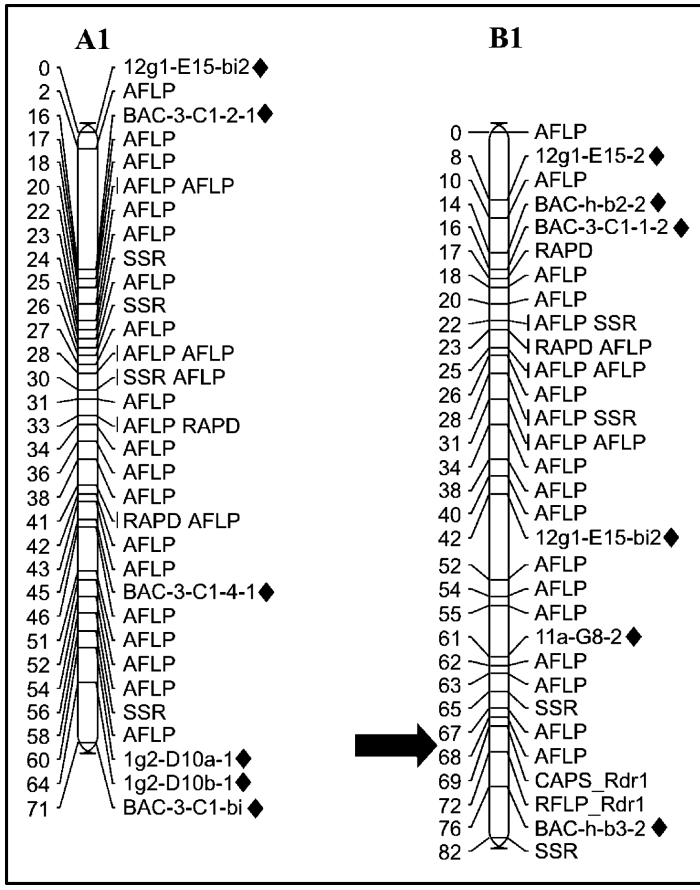

Fig. 6.4. Linkage groups 1 of the rose chromosome map for population 94/1, shown separately for the female (A1) and the male (B1) parent. The black arrow indicates the position of the black spot resistance gene *Rdr1*. Black diamonds indicate the position of resistance gene analog (RGA)–derived markers. (*Source*: Courtesy of Anja Hattendorf, University of Hannover, Germany.)

65% to 80% of genetic variation for powdery mildew resistance. One QTL, located at the top of linkage group 6, was detected in a controlled inoculation with powdery mildew race 9.

The most advanced map of rose to date includes AFLP markers as well as RFLP markers and other types including 74 SSR, 24 protein kinase (PK), and 51 resistance gene analog (RGA) markers (Yan et al., 2005). Some clustering of RGA sequences was observed, and RGAs were found mostly on linkage groups 1, 2, 4, 5, and 7 (Fig. 6.5). Linkage

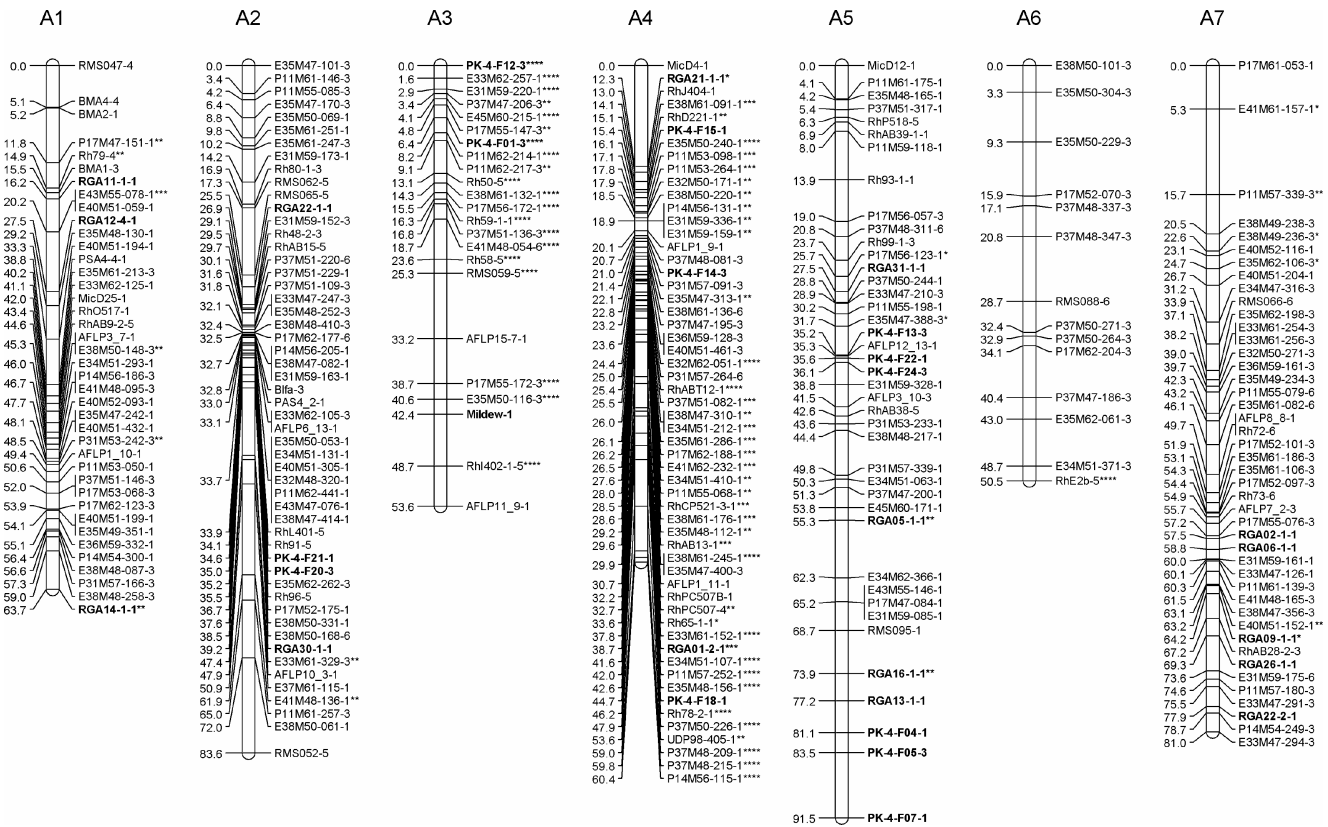

Fig. 6.5. Genetic linkage maps of diploid rose parents P117 (A) and P119 (B). Disease-resistance loci, protein kinase (PK) markers, and resistance gene analog (RGA) markers are indicated in bold.(*Source*: Courtesy of Oene Dolstra, Wageningen, The Netherlands.)

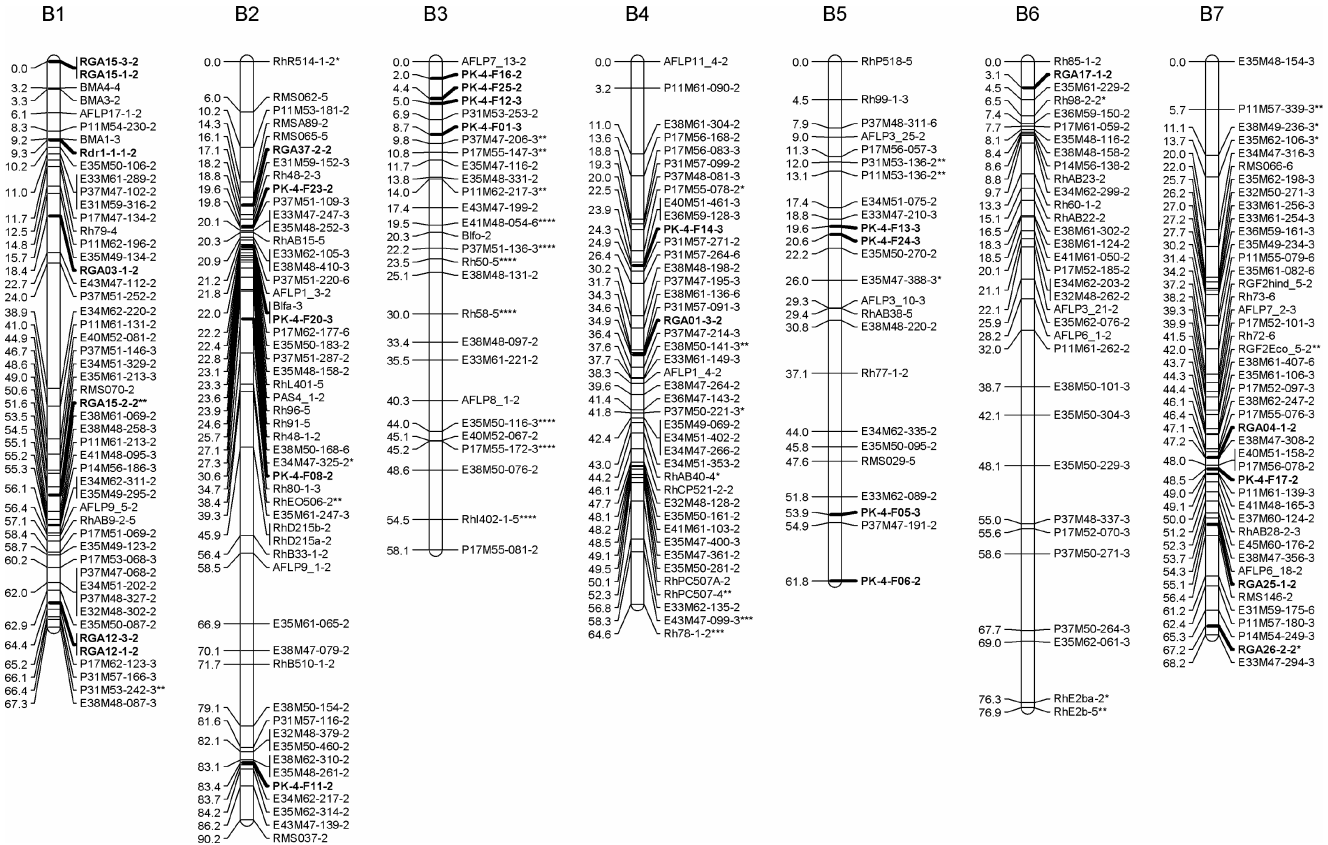

Fig. 6.5. (*Continued*)

group 6 contained only one RGA marker and no PK markers. Importantly, the SSR and RFLP markers will serve as anchor points for integration of this map with other rose maps. As marker and map information for *R* genes and QTLs increases, the feasibility of marker-assisted selection will also increase. Two additional rose maps have been constructed that do not include map locations for resistance genes but that may help facilitate future efforts. Crespel et al. (2002b) evaluated the traits of recurrent bloom, double flowers, and thorn density in a population of diploid roses using AFLP. Despite the complexity of inheritance in tetraploids, Rajapakse et al. (2001) constructed a low-density map from a tetraploid F_2 population, which may serve as a useful starting point for the mapping of important traits in cultivated roses.

Marker information can be used to select for desirable genomic regions; it also can be used to select against undesirable genomic regions. Debener et al. (2003) demonstrated this idea in the context of breeding for black spot resistance by using AFLP markers in a backcrossing strategy to select against the genome of the wild donor species (*Rosa multiflora*) in progenies segregating for *Rdr1*. The species (donor) parent and the cultivated parent initially were screened for genotype-specific markers. Plants of the BC_1 generation containing *Rdr1* were tested again for the presence of these markers. These plants segregated for 32% to 82% of the donor genome. Plants with the least amount of the donor genome were selected as parents for the next backcross generation. Molecular marker screening proved to be more effective than morphological screening for reduction of the wild species' genetic background in both the BC_1 and BC_2 generations. Using markers for efficient backcrossing may allow breeders to reduce the number of backcrosses required to eliminate undesirable alleles, thereby saving time and expense (Debener 2000).

B. Candidate Gene Approaches

Major resistance genes (*R* genes) encode proteins that function in pathogen perception, acting as receptors (either directly or indirectly) for products of pathogenic avirulence (*Avr*) genes. The combination of *R* and *Avr* genes in the host and fungus determine the race specificity of the interaction. By examining the DNA sequence of *R* genes from various plant species, researchers have gained insights into the structure of these genes. This knowledge enables the discovery of new genes. By far the most abundant class of *R* genes is the NBS-LRR class, so named for its nucleotide binding site (NBS) domain and its C-terminus

leucine-rich-repeat (LRR) domain. The NBS may be involved in signal transduction and contains several conserved motifs; the LRR apparently functions in pathogen recognition (Young 2000). Many of these proteins contain an N-terminus Toll/interleuikin receptor (TIR) domain. The non-TIR class of NBS-LRR proteins may contain coiled-coiled (CC) or leucine-zipper (LZ) motifs. Although sequences of NBS-LRR genes may be quite divergent, the presence of highly conserved amino acid motifs within the NBS domain allow the use of a degenerate PCR approach in isolating these sequences (Leister 1996; Pan et al. 2000), which are commonly called resistance gene analogs (RGAs).

Xu et al. (2005) recently employed degenerate RGA primers for the study of powdery mildew resistance in chestnut rose (*Rosa roxburgii*). Different sets of primers were found to preferentially amplify the TIR class and the non-TIR class from a resistant parent. In all, 34 RGAs that contained continuous open reading frames (ORFs) were cloned and sequenced. A subset of these sequences were converted into sequence tagged sites (STS), cleaved amplified polymorphic sequence (CAPS), and RFLP markers and used in a bulk segregant analysis to identify linked markers in an F_1 population. These markers were then used to characterize the entire F_1 population ($n = 109$). Segregation of resistance indicated the presence of more than one gene. Three markers were found to be linked to a gene (*CRPM1*) that explained 72% of the variation for resistance.

Sequences from cloned RGAs may be used to develop molecular markers, as in Xue et al. (2005), but an alternative strategy called NBS-profiling targets *R* genes while at the same time generating polymorphic markers (Van der Linden 2004). This AFLP-like approach utilizes digestion and ligation steps, followed by two PCR steps using a degenerate RGA primer and an adapter primer. The goal is to anchor one end of the marker within a conserved motif of an *R* gene NBS region while generating marker diversity based on length polymorphisms and sequence polymorphisms within restriction sites. The NBS-profiling strategy was used to develop RGA markers for the rose map constructed by Yan et al. (2005).

A much more extensive survey of RGAs from multiple rose genotypes was conducted using seven degenerate primer pairs to amplify NBS-LRR sequences from both genomic and cDNA (Hattendorf and Debener 2007). Three primer pairs specifically amplified TIR RGAs, three primer pairs amplified LZ RGAs, and one primer pair amplified both types. A library of 7,000 clones was constructed and selected RGAs were sequenced. Sequences with at least 80% overall sequence similarity

were grouped into the same families, and 40 different families were identified. Southern blot analyses showed that RGAs were organized genomically into single-copy, low-copy, and multicopy loci. In one diploid population (97/7), 37 RGAs are located on a single linkage group, while other linkage groups contained no RGAs (Hattendorf et al. 2004). Clustering of *R* genes is a common phenomenon in plant species. In another population (94/1), several RGAs are scattered on linkage group one, some proximal to *Rdr1* but none tightly linked (Fig. 6.4). Expression studies showed the upregulation of some RGAs in response to black spot attack, but further experiments will be needed to identify which are functional RGAs (Hattendorf and Debener 2007). This survey demonstrates the breadth of RGA diversity in the rose genome and provides valuable sequence resources for construction of more PCR primers that target rose RGAs.

Xu et al. (2007) applied the degenerate PCR approach to find candidate genes coding for members of the PR-2 and PR-5 families. PR-2 genes code for β-1,3-glucanases; PR-5 genes code for osmotin. Based on single nucleotide polymorphisms (SNPs) among gene family members, single nucleotide-amplified polymorphisms (SNAP) markers were developed. One marker was linked to a minor QTL that accounted for 12% of variation in powdery mildew resistance. This approach holds promise for developing markers for other classes of PR proteins, including the PR-1 and PR-3 families, which are already known to exist in rose (Suo and Leung 2002a).

C. Other Approaches

1. Transformation. Transformation of roses has been accomplished with both particle bombardment and *Agrobacterium tumefaciens* followed by regeneration via somatic embryogenesis. Since somatic embryogenesis in roses is time consuming, development of transgenic plantlets requires a minimum of a year. Nevertheless, methods of transformation are being improved, and transgenes with antimicrobial properties already are being utilized in a research context (Dohm 2003). The first transgene for disease resistance in rose was a rice chitinase gene under the control of the CaMV 35S promoter that was transformed into ‘Glad Tidings’ using a biolistic approach (Marchant et al. 1998). For the best-performing transformant, black spot lesion diameters were reduced by 43%. Among transformed plants, the level of chitinase activity explained 96% of the variability in black spot lesion diameter. Reduction in lesion diameter was most pronounced at later stages of

infection. Since this Class I chitinase is thought to be produced in the vacuole, activity against penetrating fungal hyphae is presumed to increase with increased fungal invasion.

Agrobacterium-mediated transformation was employed to transform 'Pariser Charme' and 'Heckenzauber' with other PR protein genes, namely a Class II chitinase, a β-1,3-glucanase, and a Type I ribosome-inhibiting protein (RIP) from barley (Dohm and Debener 2001). Expression of the chitinase and glucanase in the cytosol had no effect on black spot infection rates, but apoplastic accumulation of the RIP protein decreased infection by 40% on average. Expression of a T-4 lysozyme had no effect. Somaclonal variation during regeneration produced some morphological deviation and individuals with reduced fertility. Marchant et al. (1998) recovered only phenotypically normal plants, possibly because the callus phase was shorter for biolistic transformation.

Li et al. (2003) transformed 'Bucbi' (Carefree Beauty™) with a different PR protein gene in the defensin class, namely a cysteine-rich antimicrobial protein (AMP) gene called *Ace-AMP1* that originally was isolated from onion seeds. Out of seven transgenic roses, six showed decreased hyphal spread and sporulation of *S. pannosa* in both detached leaf and whole-plant inoculations. Powdery mildew symptoms were decreased by approximately 50% for the most resistant transformants. Transformation of scented geranium with the same gene, *Ace-AMP1,* reduced botrytis blight symptoms, suggesting that this gene has potential to confer resistance to *B. cinerea* in rose, although this hypothesis has not yet been tested (Bi et al. 1999).

2. Somatic Hybridization. Another technology for the introduction of disease-resistance genes is somatic hybridization through protoplast fusion, which has potential for transfer of resistance from species into susceptible cultivars or for introgression of nonhost resistance from other genera in the *Rosaceae*. Successful protoplast fusion was demonstrated in Mottley et al. (1996) by the self-fusion of a *Rosa persica* × *xanthina* hybrid. Resultant plants were doubled from the diploid to the tetraploid level, which was confirmed by chromosome counts as well as guard cell lengths and chloroplast numbers. In a later study, protoplast fusions for the purpose of disease resistance were carried out between rose species and cultivars (Schum and Hofmann 2001). Fused calli were obtained for hybridizations of 'Heckenzauber' + *R. wichuriana* and of 'Pariser Charme' + *R. wichuriana*, which were confirmed by AFLP analysis. However, at the time of publication, no plantlets had been regenerated successfully from the hybrid calli.

Squirell et al. (2005) completed the next step of producing putatively intergeneric cell lines of *Rosa* + *Prunus*, which was confirmed by RAPD analysis, although regenerated plantlets contained RAPD markers from *Rosa* alone. *Rosa* + *Rubus* fusions were also performed that resulted in plants with phenotypes strikingly different from *Rosa*, although once again RAPD markers could not confirm their status as intergeneric hybrids. It was suggested that asymmetric fusion, which assimilates only a part of the non–rose donor genome, might prove more successful. Since crosses of roses with other genera within the *Rosaceae* have not been successful, and since transgenic roses may encounter political and social obstacles, protoplast fusion has advantages as a breeding technique (Squirell et al. 2005). However, more work is needed to refine current protocols and to identify genotypes with increased capacity for regeneration (Schum and Hofmann 2001).

VI. FUTURE PROSPECTS

During the last two decades, knowledge of the general biology, diversity, and culture of rose pathogens and host mechanisms of resistance to these pathogens has greatly increased. Building on this foundation, genetic studies of resistance have begun in earnest. The discovery and analysis of race-specific genes for black spot (*Rdr1*) and powdery mildew (*Rpp1*) are an excellent start, but they comprise only a small fraction of the race-specific factors that are known based on race-test comparisons of rose genotypes (Debener et al. 1998; Linde and Debener 2003). Therefore, the door is open for further genetic characterization of race-specific resistance for black spot and powdery mildew. An even wider door exists for genetic studies of resistance to other fungal diseases such as downy mildew and spot anthracnose. And nothing is known about the inheritance of viral resistance in roses or even methods for scoring virus symptoms.

Once multiple genes against any pathogen have been identified, they may be used in a pyramiding strategy to gain broad resistance. A gene pyramiding scheme for resistance has not yet been demonstrated for rose, either for major genes or QTLs. In order to explore the diversity of resistance genes for any pathogen, it will be necessary to more firmly establish the diversity of pathogenic races worldwide. Since race collections of black spot have already been established and maintained on two continents, *D. rosae* is probably the first rose pathogen for which a standard host differential set will be established (Debener et al. 1998; Yokoya et al. 2000; Whitaker et al. 2007c).

Less is known about the genetics of partial resistance, although it generally appears to be polygenic. Isolate-specific affects on levels of partial resistance and an apparent isolate-specific QTL for powdery mildew demonstrate the importance of determining race-specific effects (Linde et al. 2006; Yan et al. 2006). Prior knowledge of the isolate specificity of QTLs would increase the potential effectiveness of a marker-assisted breeding strategy. Examining the inheritance of partial resistance using defined pathogenic isolates would be advisable, whether conducting QTL studies or other quantitative analyses. For practical purposes, breeders are concerned with determining which genotypes are useful parents for resistance. Combining ability studies based on progeny means are useful for such determinations and could be conducted. For example, some apple cultivars have been identified as desirable parents because they possess general combining ability for resistance to powdery mildew (*Podosphaera leucotricha* Salm.) (Bus et al. 2005).

As markers and maps continue to be developed for other important crops of the Rosaceae, rose breeding programs can take advantage of this genomic synergy. Comparison of *Prunus* (almond, cherry, peach, etc.) and *Malus* (apple) maps reveals consistent marker order and a high level of synteny between their genomes. Smaller regions of conservation even were found between *Prunus* and *Arabidopsis* (Dirlewanger et al., 2004). Of 46 gene-specific primer pairs (20–24 bp) representing 18 genes from *Fragaria* (strawberry, subfamily *Rosoideae*), over half amplified PCR products of the expected size from *Malus* (subfamily *Maloideae*) and *Prunus* (subfamily *Prunoideae*) (Sargent et al. 2007). Transfer of markers from *Fragaria* to *Rosa* should be at least as efficient, if not more efficient, since *Fragaria* and *Rosa* are both part of the *Rosoidae*. As markers for disease-resistance genes from other rosaceous crops continue to be identified, they could have application for rose breeding via comparative mapping. Marker-assisted selection for pyramiding of resistance genes and early selection is being applied increasingly in apple for powdery mildew resistance and other traits (Dirlewanger et al. 2004). As genomic resources for rose and for other rosaceous crops such as transferable SSRs, expressed sequence tags (ESTs), microarrays, and physical maps continue to accumulate, candidate gene approaches also will become more widespread.

Although rose has a shorter generation time than most woody crops, it is still very long compared to many crop species. A transgenic approach to disease resistance could be advantageous in that it would reduce the amount of crossing and backcrossing necessary to obtain resistance in highly cultivated forms such as hybrid teas. However, the advantages

and disadvantages must be weighed carefully. The process of transformation, regeneration of desirable transgenic lines, and field testing of transgenics may be no less time consuming and expensive than traditional breeding approaches. Through asexual propagation, desirable transgenic lines of roses could be increased quickly for commercialization. Since rose (except in the context of rose hips) is not a food product, the public may be more amenable to transgenic roses in light of the environmental benefits of disease control not involving pesticides. Overexpression of PR proteins from other species has been demonstrated to confer partial resistance phenotypes to fungal diseases in garden roses. In addition, these resistances have the potential for effectiveness against a broad spectrum of pathogens. However, a difficulty with this approach is that many potential candidates from other crops are protected by patent law and may be difficult or expensive to utilize commercially (Dohm 2003). Since the presence of PR proteins such as β-1,3-glucanase and chitinase has been detected in rose (Suo and Leung 2001a), this problem possibly could be avoided by cloning these genes from rose and overexpressing them in transgenic lines. Indeed, β-1,3-glucanase and osmotin genes already have been cloned from *R. roxburghii* (Xu et al. 2007). Major resistance genes also would be good candidates for a transgenic approach if their race specificity is well characterized and if they were used wisely. In the future when multiple *R* genes have been cloned from rose, these could be introduced simultaneously into susceptible varieties to achieve broad resistance to multiple pathogens and/or multiple pathogenic races.

Transgenes designed to induce RNA interference (RNAi) have been introduced successfully in other plant species to confer resistance to crown gall and root-knot nematode and could also be applied to rose. Tumorigenesis in plant roots infected by *A. tumefaciens* is dependent on the oncogene region of the bacterial T-DNA. Transgenic *Arabidopsis thaliana* and *Lycopersicon esculentum* (tomato) carrying constructs for expression of double-stranded RNA (dsRNA) effectively eliminated expression of the *iaaM* (auxin production) and *ipt* (cytokinin production) oncogenes, thereby halting tumorigenesis (Escobar et al. 2001). This method also has been used to generate crown gall–resistant apple trees and is expected to be a highly durable resistance mechanism (Viss et al. 2003). Resistance to root-knot nematodes has been achieved in *A. thaliana* through transformation with a dsRNA corresponding to a nematode parasitism gene (Huang et al. 2006). After piercing the transgenic plant root with its stylet, the nematode ingests the dsRNA and the nematode parasitism gene is silenced. Because the target gene is highly conserved and is essential for pathogenesis, the

transgenic plants were resistant to four different *Meloidogyne* species (including the important rose pathogen *M. hapla*). Such a breadth of resistance to root-knot nematode had never been achieved using natural genetic resistances.

Genomic approaches utilizing marker-assisted selection and transgenes will not replace but rather complement traditional breeding for disease resistance in roses. Resistance is available in the species germplasm and also among cultivars. Although acquisition of wild germplasm will continue to be important in rose breeding, characterization of the race specificity and genetic transmission of existing resistances is of first importance. This will be accomplished through more comprehensive pathotype characterizations, reliable disease screening methods, and genetic studies. Continued refinement of polyploidization methods also could improve resistance breeding. The use of molecular markers to eliminate nondonor contributions and to select for resistance genes in the context of breeding programs is promising. The decreasing cost of marker technology and other molecular tools along with public demand for nonchemical disease control will mean that conventional strategies and genomic approaches will progress hand in hand. Rose breeders will continue to glean new information from advances in other crops that ultimately will result in disease resistance that is effective and long lasting.

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