CHAPTER 5

# Naturalization of Host-Dependent Microbes After Introduction into Terrestrial Ecosystems

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"Understanding how genotype and environment interact to determine an organism's phenotype and fitness is a fundamental goal at the interface of ecology, genetics, and evolution" Remold and Lenski (2001)

## Introduction

Introduction of plant pathogens, insects, parasites, and predators into terrestrial and marine ecosystems is second only to habitat loss among major threats to biodiversity (Torchin *et al.* 2002), and the frequency of introductions continues to increase (Flather *et al.* 1998, Torchin *et al.* 2002, Wilcove *et al.* 1998). Despite their detrimental impacts, introductions can also be seen as massive experiments having a defined time of onset, and the possibility to define and monitor changes in genetic structure of both host and parasite across environments over time. As such, introductions of parasites and pathogens are a potentially rich source of fundamental knowledge about host-parasite interactions. These introductions initiate a nexus of interactions at the interface of ecology, genetics, and evolution.

Achieving the greatest understanding of interactions between introduced pathogens and hosts requires cross-disciplinary approaches. Such approaches will require the integration of multiple fields both between and within the disciplines (e.g., plant and animal pathology / parasitology) and the development of either common terminology or sufficient "cross-talk" to allow interpretation of concepts. These needs have been repeatedly recognized: In a recent review, Milgroom (2001) considered that the field of plant pathology would benefit from greater unification of the topics of population genetics and epidemiology. Galvani (2003) made a similar plea to "...merge population genetics and epidemiological dynamics of transmission..." to provide better management of human diseases. Plant and animal pathology must also consider whether our understanding of the operation of traits affecting host-parasite interactions and epidemics is transferable from natural to "artificial" pathosystems and *vice versa*. The study of natural ecosystems and application of natural system-based models to investigate disease in agroecosystems was addressed in an American Phytopathological Society symposium in 1974 (McDonald 1974). Based on wild cereal rust pathosystems in Israel, Browning (1974) listed six mechanisms that balance the amount of disease in host-pathogen populations. These included general non-host immunity, durable resistance, R-gene resistances, tolerance, antagonists, and complex host-pathogen population interactions.

In a pivotal review of disease in wild plant populations, Jarosz and Davelos (1995) attempted to forge a connection between the plant and animal / medical disease literature. A serious problem then and now is that the term "virulence" is applied differently in the two disciplines. In the non-plant literature, virulence generally refers to the ability to reduce host survival and reproduction, whereas plant literature typically refers to virulence as a pathogen trait for compatibility between pathogen and host that allows initial infection to occur. Jarosz and Davelos (1995) attempted to solve the problem by equating the use of the term "aggressiveness" in plant pathology with use of the term "virulence" in other disciplines. They then attempted to examine the evolution of virulence (the broader sense, as also used throughout this chapter) from the perspectives of both plant and animal epidemiological literature.

Aspects of plant and animal epidemiology have also been compared by Waggoner and Aylor (2000). They contend that medical epidemiologists focus on understanding how disease processes occur in populations of host and parasites, whereas plant epidemiologists focus primarily on management of disease while ensuring minimal environmental impact. On this basis, these authors conclude that medical epidemiologists may be better "disease detectives" than plant epidemiologists. In their look to the future, they consider invasions to constitute the largest disease

threat, and challenge plant epidemiologists to become better disease detectives.

Evolutionary epidemiology, a key discipline in medical epidemiology (Ewald 1994, Ewald *et al.* 1998), might help plant epidemiologists become better disease detectives. An amalgamation of ideas potentially useful to phytopathologists has begun within the community of evolutionary biologists and medical epidemiologists that study host-parasite interactions ranging from phage-bacteria interactions through plant-insect to parasitic plant-plant interactions (Day 2003, Ebert and Bull 2003, Little 2002, Slev and Potts 2002). These and related investigations seek to merge epidemiological theory and evolutionary biology of hosts and parasites (sometimes also referred to as "victims" and "exploiters", respectively in this literature) into the field of evolutionary epidemiology. For example, Bergelson *et al.* (2001) provided insight into complex interactions among ecological, evolutionary, and epidemiological processes that underlie the *evolution of virulence*. A central concept was that evolution of virulence is mediated by tradeoffs in life history that are also associated with different traits of exploiter virulence (capacity to cause mortality or otherwise impact host fitness) and exploiter reproductive / transmission success. This concept has also appeared in the phytopathological literature (Thrall and Burdon 2003, Zhan *et al.* 2002). Our goal in this review is to further apply tenets of evolutionary epidemiology to the analysis of a classic parasitic invasion into a terrestrial ecosystem – white pine blister rust in North America – to demonstrate how the application of a general theory of host-pest interaction can create fresh perspectives, increase analytic power, and focus new research into landscape perspectives on disease.

## Evolutionary Epidemiology

We propose four key principles central to evolutionary epidemiology: First, natural systems having a resistance genetic structure are characterized by unevenness in occurrence of infections, i.e., the epidemiological phenomenon known as "overdispersion". Second, the evolution of virulence is constrained by tradeoffs in relative advantages of different life-history traits. Third, the combination of resistance genetic structure in host and virulence structure in pathogen constitutes a local interaction structure that can vary with time and space. Fourth, phenotypic plasticity can allow for different expression of the genetic makeup of hosts and pathogens in different environments.

Before applying these four principles from evolutionary biology and medical epidemiology to the disease dynamics of specific plant pathosystems, it is useful to consider examples of the inherent but changing assumptions on which epidemiological models have been based in animal vs. plant pathosystems. Three such comparative assumptions include the following: 1) According to plant disease researchers Jarosz and Davelos (1995), the number of animal hosts ultimately limits the pathogen population size in animal models, whereas climate, including weather and growing season, are limiting to plant diseases. 2) Another common assumption of animal epidemiology had been that an individual host cannot become infected with more than one strain of a pathogen; however, this assumption is no longer held to be true (Van Baalen and Sabelis 1995). 3) The most critical assumption of the animal models is that a trade-off between aggressiveness and transmissibility of the pathogen will drive evolution toward an optimum level of aggressiveness or virulence. In contrast to animal systems, Jarosz and Davelos (1995) concluded that even though plant diseases exert major influences on plant fitness, little evidence could be found for evolution of reduced virulence (aggressiveness). Recent studies can be interpreted as supporting (Thrall and Burdon, 2003) or conflicting with (Zhan *et al.* 2002) the findings of Jarosz and Davelos (1995). However, a possibility not considered by these authors is that genetic variation for life-history traits in plant pathogens could directly or indirectly influence realized virulence (mortality of hosts or host parts), thereby imposing strong selective forces on pathogen strains. Because genetic variation in life-history traits appears to be a real possibility in some plant pathosystems, we will reinvestigate the value of models for evolution of virulence in pathogens for understanding disease in wild plant populations.

**Overdispersion.** Disease epidemics in plants are usually measured in terms of incidence (proportion of a population infected) and severity (average number of infections per host). The accumulation of infections on individual hosts and stands is influenced by density and distribution characteristics of pathogen propagules, their inoculum potential (ability to infect), the amount of host target, and the biology of the pathogen (e.g., new cycles of infection at one or repeated times in a season for monocyclic and polycyclic diseases, respectively) (Madden and Campbell 1990). A plot of incidence versus time produces the disease-progress curve (Madden and Campbell 1990). The shape of the disease-progress curve is determined by the time-dependent accumulation of infections, and its asymptote is the maximum level of incidence that the stand should reach. The severity / incidence (S-I) relationship is independent of elapsed time (Seem 1984). Yet, it will reveal the same asymptote as the disease-progress curve.

In earlier models, random distribution of pathogen propagules was assumed within a population of hosts, so that existing infections would have no influence on the probability of which hosts would be infected in future infections. Under this assumption, the Poisson distribution would be an adequate model to describe the S-I curve (Fracker 1936, Waggoner and Rich 1981). However, "overdispersion", or unexpected aggregation of infections on certain hosts but no infections on others, is a remarkably common feature of almost all host-pest interactions, from nematodes on insect hosts (Westerman 1999) to deposition of blowfly eggs on sheep (Fenton *et al.* 1999). Most plant pathogens also

exhibit "overdispersion" (Waggoner and Rich 1981), and S-I curves generally conform to the negative binomial distribution (NBD) instead of the Poisson. A fit to the NBD, but not to the Poisson, can indicate that infections are not distributed randomly, so that among other factors, past infections could be influencing the probability of new infections.

One fundamental cause of non-random dispersion of infections on hosts is genetic interaction among hosts and pests. Genetic interaction can take the form of R-genes (qualitative resistance), r-genes (quantitative resistance genes that reduce rate of infection and/or aggressiveness of pathogen development), and various kinds of induced host responses that can clear the host of the pathogen or prevent new infections. Both plants and animals have the ability to distinguish infectious non-self from noninfectious self (Janeway and Medzhitov 2002). Animals express both adaptive and innate immune responses, whereas plants express only innate responses (Janeway and Medzhitov 2002).

NBD can result from an interaction of several Poisson distributions. For example, if a host population were composed of four classes of plants, each might have a unique rate of disease incidence with a Poisson distribution, but the population's distribution would still fit the NBD. Overdispersion that causes a fit to the NBD might also be due to spatial differences unrelated to resistance that affect inoculum production or distribution, such as heterogeneous distribution of localized inoculum or microclimate during infection episodes, or a combination of these sources.

**Evolution of virulence.** At the center of our discussion about evolution of virulence is tradeoff theory. (To reiterate, we are considering virulence in the broad sense: the capacity to cause mortality or otherwise decrease fitness of the host.) Negative correlations between virulence and other traits important to pathogen fitness create an evolutionary tradeoff between rapid reproduction and transmission from an infected host vs. maintenance of long-term infections that consequently allow long-term transmission from the host (Lipsitch and Moxon 1997). According to this model, pathogen evolution will move toward a level of virulence where its basic reproductive rate will be maximized (Lipsitch and Moxon 1997). Basic reproductive rate is taken as the average number of new infections caused by a single infectious host in a previously uninfected population. Models that are more complex can consider the influences of host population structure (e.g., quantitative and "strain-specific" resistance); horizontal vs. vertical transmission of infections (i.e., between vs. within individuals); infection by multiple pathogen strains; roles of vectors (including alternate hosts); presence and action of host "clearance" of infections through acquired, induced, or developmental resistance; and host life history and behavior .

Pathogen virulence comes under renewed selective pressure whenever the tradeoff between parasite virulence and success of parasite transmission to new hosts has been altered. When a pest is introduced to a new host, a new host is in a new environment, or an established host and parasite are introduced to a new environment, disease or virulence may increase (Ebert 1998). As demonstrated by experiments with animal parasites serially passed through hosts, such systems can evolve rapidly (Ebert 1998).

According to the tradeoff model, pathogens will evolve toward a level of virulence that maximizes their basic reproductive rates (Lipsitch and Moxon 1997). Thus, the parasite attains an evolutionarily stable adaptive "peak" that results in an intermediate or even low level of virulence. The mechanisms by which rapid and local evolutionary responses in virulence occur in a parasite / pathogen / predator may or may not be attributable to changes in gene frequency (classic evolution), as organisms can also have changes in gene expression (phenotypic plasticity, described below). Such changes may be the foundation of most victim-exploiter interactions (Baldwin 2001, Tollrian and Harvell 1999).

**Formation of a local interaction structure.** Wild host-parasite couplets have a local coevolved structure characterized by a dynamic equilibrium that allows simultaneous survival of host and pest. Such coevolved structures likely involve evolutionarily stable life-history strategies that maximize reproductive success within the constraints of specific environments. This finding has supported interest in the theory of local adaptation (Gandon and Van Zandt 1998, Kaltz and Shykoff, 1998, Little 2002, Mopper 1996, Van Zandt and Mopper 1998), metapopulation dynamics, and evolution of maladaptation (Crespri 2000). Two significant generalizations have been suggested for diseases in natural ecosystems, as follows: 1) Expression of disease in wild ecosystems is highly variable in time and space; and 2) Patterns of strong local adaptation develop rapidly, with hosts and pathogens developing classic metapopulation structures by the combined natural forces of selection and gene flow (Burdon and Thrall 1999, Dinoor and Eshed 1984, Gandon and Van Zandt 1998, Gilbert 2002, Heath 1991, Jarosz and Davelos 1995, Kaltz and Shykoff 1998, Thompson 1999a, 1999b, Thrall and Burdon 1997). In the last few years, the geographic mosaic theory of coevolution (Thompson 1994) has been used to examine dynamics of host pest coevolution in wild populations (Thompson 1999b, Thrall and Burdon 1999, Thrall *et al.* 2001). This theory presupposes geographic mosaics that cause "hotspots" of selection and coevolution (Thompson 1997, 1999a, 1999b), linked to the dynamics of local metapopulations (Hanski and Gilpin 1997).

Other studies of evolutionary processes have used artificial selection and changes over time or have used adaptation as a geographic pattern (Kaltz and Shykoff 1998). These concepts have been based almost entirely on information from annuals and long-lived hardwood hosts, with little recognition of the extensive literature on conifer hosts. Yet, host life-history traits and generation time are known to be important factors in this evolutionary process (Kirchner and Roy 1999), and knowledge about disease in natural, conifer-dominated ecosystems would benefit from consideration of metapopulation theory (Lundquist and Klopfenstein 2001). In addition, almost all of the literature

regarding local coevolutionary processes between hosts and pathogens has concerned simple, gene-for-gene models having clearly defined R-genes in hosts and corresponding avirulence genes in the pathogens. Furthermore, most studies rely on phenotypic expression of disease in specific host-pathogen combinations to imply genotype. Undoubtedly, classic gene-for-gene interactions play important roles in wild pathosystems, but partial resistance may be of equal or greater importance, as has been documented for some interactions involving viruses, fungi, helminthes, protozoa, and herbivores (Ebert and Hamilton 1996). Some examples that illustrate local structure in woody plant systems are pinyon pine / stem moth (Mopper *et al.* 1991), oak / leaf herbivore interaction structure shown by reciprocal transplant experiments (Sork *et al.* 1993), blister rust interactions shown by reciprocal transplant of *Ribes* (Kimmey and Mielke 1944), and *Ribes*-blister rust cross inoculation experiments (McDonald 2000).

**Phenotypic plasticity.** Phytopathology, as an applied discipline with a deep foundation in ecology, genetics, and evolution, has a fundamental interest in understanding genetic x environment (G x E) interactions. Influences of mutations, hybridization, founder events, and gene regulation on G x E interactions are core issues that must be addressed to understand adaptation of introduced pests in natural ecosystems as well as the ongoing selective processes that occur in highly managed agroecosystems. Evolutionary biology, where the disciplines of quantitative genetics, molecular genetics, evolutionary ecology, systematics, and paleontology converge (Pigliucci 2001), can provide significant insights into the process of naturalization. One of the most basic questions in biology is that of "nature vs. nurture". Over the past two decades, the respective role of traditional genetic factors vs. the altered ability of a genotype to respond to different environments (phenotypic plasticity) has been intensively investigated in botany, zoology, and entomology. The concept of phenotypic plasticity and the reaction norm (a commonly used technique for visualizing phenotypic plasticity) are rapidly becoming tools of choice for understanding evolution (Pigliucci 2001). This concept can be applied to highly variable pathogens found in agroecosystems and, since naturalization is nothing more than a specific case of evolution, to introduced pathogens in natural ecosystems. Yet, this revolution in evolutionary biology has largely bypassed plant pathology. A search of Phytopathology Online turned up only one reference (Lorys *et al.* 2000). A search of Annual Review of Phytopathology turned up two references (Andrews and Harris 2000, Kinkel 1997). Two additional discussions of phenotypic plasticity were also found (Andrews 1991, McDonald 1996).

The phenotype of an organism is based on the expression of its genotype under particular environmental conditions. All phenotypic responses are thus dependent upon both classical genetic traits and differences in the expression of those traits due to the abiotic and biotic environment. Differences in trait expression in different environments are thus attributable to phenotypic plasticity. Moreover, the response of pathogen / parasite populations to their counterparts and to their environment during coevolution can be attributed in various degrees to both genetic selection and phenotypic plasticity.

The need to differentiate between these processes is demonstrated by a case history. Thompson (1998), in listing examples of rapid and local coevolution as important features of wild host / pest interactions, chose the rapid (i.e., within ca. 100 years), but geographically variable, morphological response of an intertidal snail (*Littorina obtuscata* L.) to the introduction of a crab predator (*Carcinus maenas* L.). The snail's response, production of a thicker shell, was originally believed to be the result of rapid coevolution fueled by intense natural selection (Seeley 1986). However, this example was subsequently demonstrated to be a classic case of "induced defense;" shell morphology was phenotypically plastic, with an alteration in gene expression triggered by the reception of a water-borne elicitor emitted from the predator (Trussell and Smith 2000).

Of two approaches that have contributed to our understanding of how genotypes express variable phenotypes over environmental gradients (i.e., phenotype-genotype mapping and reaction norms, we will utilize the reaction norm to illustrate examples of phenotypic plasticity. Reaction norms graphically display the phenotypic responses of genotypes across environments. Responses of different phenotypes may parallel each other or diverge under different environments, indicating differences in average phenotypic responses and in the relative plasticity of phenotypes across environments. Three biological mechanisms underlying differences in reaction norms have been defined (Nijhout 1999a). In the first mechanism, different environments create different phenotypes because an environmental variable (e.g., temperature, pH, salt concentration, nutrient level, hosts, etc) affects the general functioning of a biological process, such as metabolism or growth. In the second mechanism, known as allelic sensitivity, the environmental variable induces altered levels of activity of a specific gene product that contributes to a phenotype (Nijhout 1999a, Wu 1998). The third mechanism, known as regulatory plasticity, results from gene regulation, where structural genes that encode protein can be turned on or off by environmental cues (Nijhout 1999a, Schilichting and Pigliucci 1995, Wu 1998). When reaction norms appear as step functions rather than as gradual changes over a graded environmental change, they are known as polyphenisms (Nijhout 1999a, Nijhout 2003). This step function suggests that triggering thresholds have been reached that alter gene regulation. Details of gene regulation are rapidly becoming known (Bird 2002, Chandler and Vaucheret 2001, Jaenisch and Bird 2003). In fact, altered phenotypes from some causes of gene regulation plasticity may even be inherited. Several examples of resistance genes are known, in which environmentally induced changes in phenotype were observed across generations (Agrawal *et al.* 1999, Stokes *et al.* 2002).

Phenotypic plasticity can be categorized as anticipatory, passive, non-adaptive, and adaptive (Pollard *et al.* 2001). Anticipatory plasticity is characterized by response to specific cues such as shifts in red:far-red ratios that signal changes in light quality in a plant's environment (Schmitt *et al.* 1999) and predator effluent that induces morphologic changes in tadpoles (Relyea 2002). Passive plasticity is generally characterized by a change that is proportional to the environment (Pollard *et al.* 2001). In addition, phenotypic plasticity can be adaptive or non-adaptive. Phenotypes are adaptive when their induction has higher fitness in the inducing environment, whereas non-adaptive phenotypes possess lower fitness in the inducing environment.

**Phenotypic plasticity and life history polyphenisms.** Some organisms can develop into more than one stable adult form. For rusts and other fungi, as with other organisms, the question is how to define the adult form. In most organisms, the form that reproduces sexually is considered the adult and definitive state. With other organisms, such as the honeybee, reproducing individuals have different forms from non-reproducing members of a colony, although workers and queens may be genetically identical, and differentiate only as a result of differences in diet at key developmental stages (Evans and Wheeler 1999, 2001). Such cases of sexual morphs provide strong evidence of environmental influences on gene expression.

For mammals, it is believed that most developmental switches are set at birth. From that point on, set trajectories are followed. Signaling and control is now a predominantly local matter, and the regions within which cells can communicate are called development fields (Nijhout 1999b). Autonomy of emerging development fields and coordination of development among fields are critical factors, especially as the environment varies through time and space. Mammals, with their relatively stable environment of the womb, and long distance signaling via hormones, may thus have evolutionary advantages over organisms that lack these features (Nijhout 1999b).

Do fungi use long-distance signaling to affect development? At metamorphosis, many insects can switch to different development pathways (Nijhout 2003). Can we consider the initiation of different rust-spore stages as "metamorphosis"? In a sense, the organism is undergoing metamorphosis. On the question of sex, we have mentioned previously that sterile worker bees and fertile queens develop from one genotype with the difference attributable to nutrition. How does this compare to rust life cycles where the sexual stage is lost, as in microcylic life cycles? Genes need to be turned on to make worker bees (Nijhout 1999b). Do genes need to be turned on to create a microcylic rust and does turning off these genes restore a full-cycle life history? Plant rusts are known for their ability to evolve life cycles to fit the environment in which they exist (Savile 1976) and for their ability to shift from production of urediniospores to teliospores under conditions of plant stress (e.g., cold, desiccation, and low light) or plant resistance (Waters 1928). *Cronartium ribicola* exhibits a number of different life-history modes. For example, some variants cycle to alternate hosts other than *Ribes* (e.g., *Pedicularis* spp.), while other variants or "sister species" are autoecious, cycling from pine to pine (Imazu and Kakishima 1995). Thus far, studies of DNA markers have not demonstrated clear differences between heteroecious and autoecious variants of the full-cycle and microcylic forms of *Cronartium flaccidum* (Moricca and Ragazzi 1998). If phenotypic differences are not attributable to differences in DNA, then variation in a trait may be attributable to events at the mRNA or protein level. Thus far, it remains undetermined whether the short-cycle, autoecious condition represents a polyphenism.

**R-genes, r-genes, and phenotypic plasticity.** The outcomes of individual host-pest interactions are initiated upon physical and physiological contact. Of critical importance for understanding processes of naturalization after introduction of a new pathogen are the relative roles of evolution (changes in gene frequency) and phenotypic plasticity (changes in gene expression or activity of gene products). The coevolutionary arms race between plant hosts and pathogens is well documented in the case of the hypersensitive response (HR) and R-genes in hosts and virulence or avirulence genes in pathogens (Bergelson *et al.* 2001). Conversely, adult-plant resistance in wheat and barley is characterized by seedling susceptibility and durability of resistance (Kolmer 1996, Line 2002). Such host resistance can be an induced response, reminiscent of the immune response in animals (Bergelson *et al.* 2001, Staskawicz *et al.* 2001a). Less well understood are the inheritance and coevolutionary relationships for durable and induced resistance and of systemic acquired resistance. The type of resistance should impose a spectrum of constraints on the evolution of virulence (Frank 2000, Kirchner and Roy 2001, 2002, McDonald and Linde, 2002, Roy and Kirchner 2000).

Evolutionary biology has already contributed greatly to our understanding of plant disease interactions and has the potential for additional contribution through application of reaction norms. Since partial resistance is known to be different from R-gene resistance in its temperature sensitivity and other aspects of interaction phenotypes in both host and pest, we constructed relevant examples of reaction norms for these phenomena from the phytopathological literature. Recently, Kim and Bockus (2003) published results from experiments designed to study the temperature sensitivity of wheat cultivars to three single-spore isolates of *Stagonospora nodorum*. Here, we use their data, presented as area under the disease-progress curve (AUDPC) to illustrate some key points and draw additional comparisons and interpretations. The foundation of reaction-norm interpretation is that exposure of a single genotype or group of related genotypes (e.g., family or population) to an environmental gradient facilitates rapid assessments of genetic and environmental influences on trait expression (see Pigliucci 2001). In our example, reaction norm patterns vary when particular combinations of host and pathogen are subject to different temperatures (Figures

5.1 to 5.4). These patterns reveal specific information regarding gene frequencies and gene expression. Such detailed information is fundamental to understanding evolutionary epidemiology of specific host-pathogen couplets. Additional insight may be gained if a host is considered as a component of the pathogen's environment. This may be of particular importance in regard to partial resistance, since events early in the interaction affect pathogen and disease development. We have included an example of reaction norms that detail growth of infection hyphae of wheat leaf rust strains in different host genotypes (Kloppers and Pretorius 1997). The reaction norm technique (Figure 5.5) clearly demonstrates classic E (environmental) and P (plasticity) variance and significant G x E interaction where the environment is a change in a host partial resistance gene. No variance could be *solely* attributed to G, as shown by nonsignificant cross-environment (CE) means (Figure 5.5).

## Invasion in an Experimental Context

A key aspect of the biology of any species is the manner in which its populations maintain themselves in heterogeneous environments. Most successful invaders had pioneering life-styles in their native habitats and were therefore well suited for invading new habitats. In this paper, we are specifically examining the mechanisms by which fungal pathogens of woody plants growing in natural ecosystems can invade by considering the general concept of invasiveness. A typical process associated with invasive success is a lag period after introduction and establishment that is followed by a dramatic spread (Sakai *et al.* 2001). At this point, the organism has become an invasive; ecological and human impacts follow. Most introductions do not reach invasive status. Those that do succeed may owe their success

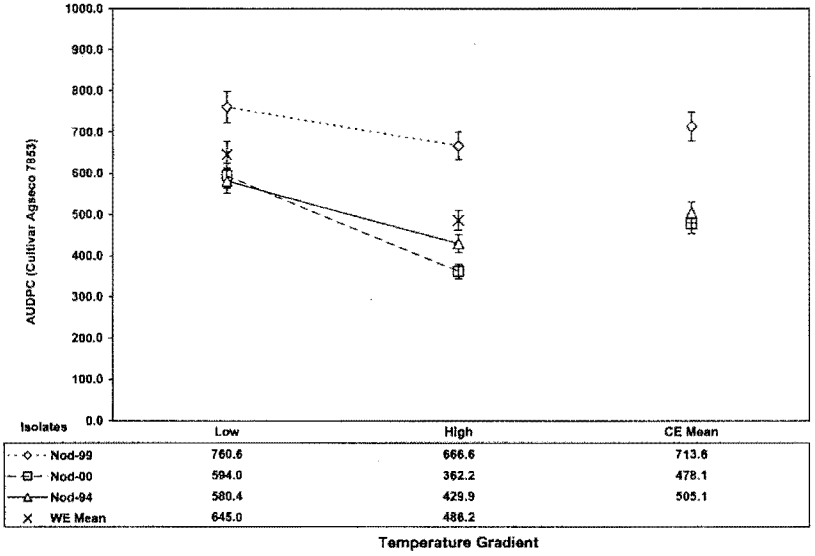


**Figure 5.1.** Example reaction norms for three single-spore lines of *Stagonospora nodorum* inoculated onto wheat cultivar Agseco 7853. Phenotypes produced under low (10-18°C) and high (21-29°C) temperatures were expressed as area under disease-progress curves (AUDPC). Data obtained from Table 2 mean of experiments 2 and 3 (Kim and Bockus 2003). Isolate Nod-99 shows genetic variance compared to Nod-00 and Nod-94. Nod-00 exhibits significant environmental variance, plasticity variance, and G x E. In this example, Nod-99 and Nod-94 show genetic variance but not G x E. Significance was judged by applying a 0.05 coefficient of variation, since standard errors were not published.

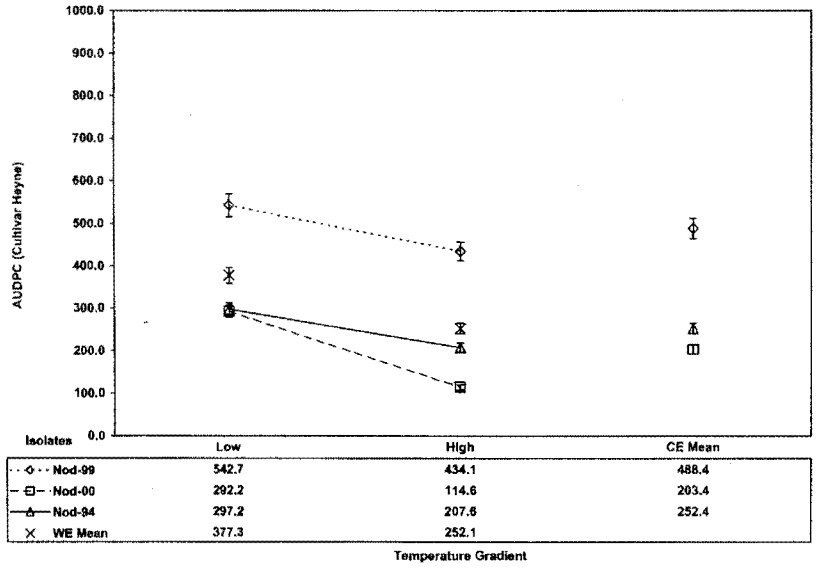


**Figure 5.2.** Example reaction norms for three single-spore lines of *Stagonospora nodorum* inoculated onto wheat cultivar Heyne. Phenotypes produced under low and high temperatures were expressed as area under disease-progress curves (AUDPC). Data obtained from Kim and Bockus (2003), and significance tests were derived as in Figure 5-1. Isolates Nod-99, Nod-00, and Nod-94 all show genetic variance. Significant environmental variance is present as well as plasticity variance and significant G x E for Nod-00. In this example, Nod-99 and Nod-94 show genetic variance but not G x E.

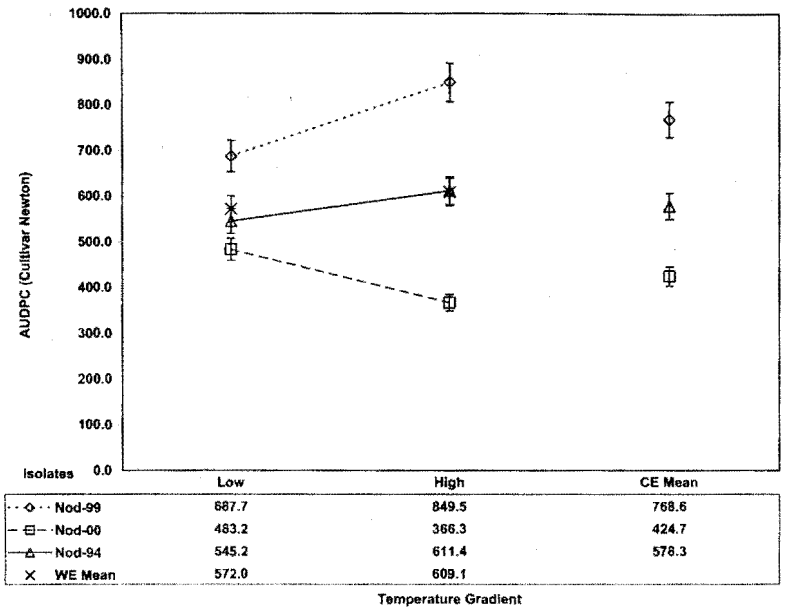


**Figure 5.3.** Example reaction norms for three single-spore lines of *Stagonospora nodorum* inoculated onto wheat cultivar Newton. Data source (Kim and Bockus 2003) and significance estimates were derived as in Figure 5-1. The pattern of expression is different on the cv. Newton. Nod-00 shows the same pattern on all three cultivars, but Nod-94 and Nod-99 shows increased disease at higher temperature on cv. Newton. E variance is not significant, Nod-94 does not exhibit P variance, but Nod-00 expressed significant G x E variance.

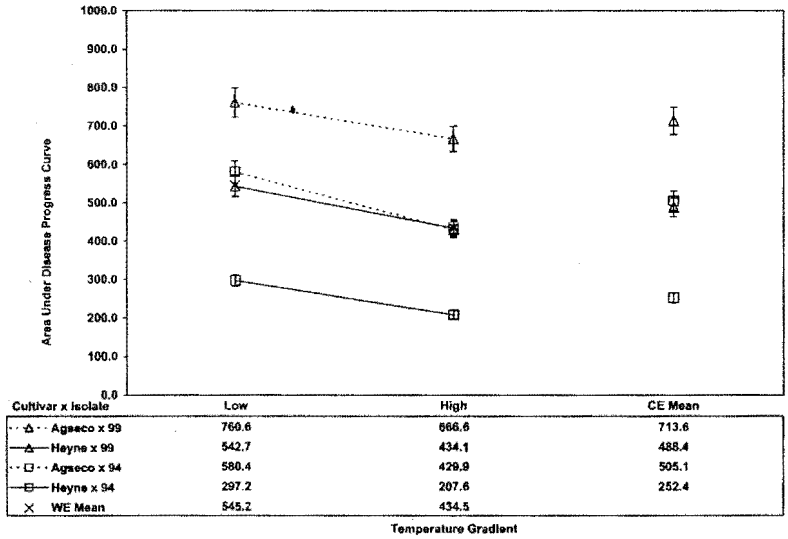


**Figure 5.4.** Example reaction norms for two single-spore lines of *Stagonospora nodorum* inoculated onto wheat cultivars Agseco 7853 and Heyne. This test is designed to compare similar reaction norm patterns shown in Figures 2 and 3. Data source and analysis were derived as in Figure 5-1 (Kim and Bockus 2003). These reactions show significant G, E, and P variance, but not G x E variance. Even though the cultivar x isolate patterns of these four combinations are nearly identical, potentially significant information is revealed by examining all reaction norm combinations. Specific combinations of host and pest indicate whether isolates can be separated by their apparent interactions with hosts carrying quantitative resistance genes.

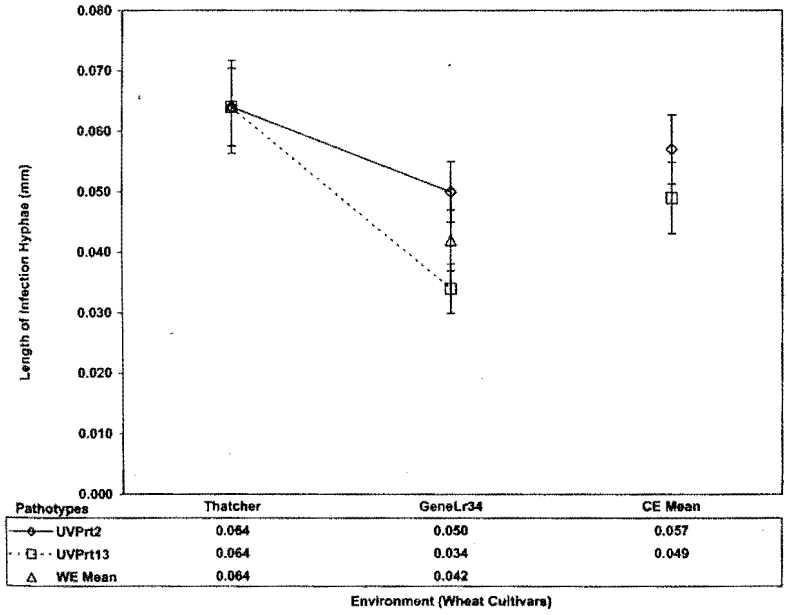


**Figure 5.5.** Example reaction norms of infection hyphae lengths for two pathotypes of leaf rust inoculated onto two cultivars of wheat (Table 2 in Kloppers and Pretorius 1997). Significance judged from standard deviations presented with data. Genetic variance of rust genotypes is nonsignificant (CE means are not different), environmental variation of rust genotypes is significant (WE means are significantly different), and differential plasticity variance is present (UVPrt2 and UVPrt13 differ significantly on wheat cultivar carrying gene Lr34). Therefore, assuming that wheat genetic constitution represents environmental variation for the rust, the rust is expressing G x E variance.

to external forces (characteristics of the new environment) and to internal factors (e.g., evolution, hybridization, founder effects, etc) (Tsutsui *et al.* 2000). Invasions favored by environmental factors, such as absence of predators, pests, or competitors or a change in abiotic environment, are usually obvious.

A theoretical framework developed to analyze successful invasions in marine ecosystems (Torchin *et al.* 2002) uses biotic interactions, life history, and demographic parameters to model how resources, competition, predation, and parasitism impact invasion success. In this framework, three measures of invasion success are: absolute success (biomass at equilibrium is a value greater than zero), relative conspecific success (biomass of the invader is greater in its introduced range than in its native range as calculated on basis of available habitat), and relative interspecific success (the invader biomass is greater than biomass of competing native species as calculated on the basis of available habitat). Measurement of invasion success requires comparison of biological and ecological behavior of target organisms in both native and invaded situations, as illustrated by the analytic study of marine invasions (Torchin *et al.* 2002).

Stages of a successful introduction have also been described by Sakai *et al.* (2001) as: 1) introduction into a new habitat, 2) successful establishment, and 3) secondary dispersal into new habitats. They also recognized that at each stage, many opportunities exist for large genetic change through genetic drift, founder effects, and selection. The three hypotheses generally advanced to explain successful invasions are 1) a better environment, 2) less competition, and 3) release from natural enemies (Torchin *et al.* 2002). Introduced species released from predation often attain larger body sizes and higher population densities compared to their native habitats (Torchin *et al.* 2002). In the case of a pathogen introduced to a new host, a new host in a new environment, or an established host-pathogen couplet introduced to a new environment, more disease or increased virulence is usually the result (Ebert 1998). Thus, a fourth stage or hypothesis should be considered for introduced parasites: the coevolution of victim-exploiter interactions.

## White Pine Blister Rust in North America

The introduction of *Cronartium ribicola* J. C. Fischer ex Rabh., causal agent of white pine blister rust (WPBR), occurred at many points in eastern North America (Spaulding 1922) and a single point (Point Grey, British Columbia, Canada) in western North America in 1910 (McDonald and Hoff 2001). This introduction initiated an evolutionary experiment of continental proportions that is now about 100 years old. Breadth of host spectrum, variation in the time that blister rust has been at different locations, and the variety of locations and environments of establishment makes WPBR an ideal example for the study of many aspects of pest naturalization. The introduction of WPBR to North America involves the botanical ranges of nine pine hosts, a large number of alternate host species belonging to the genus *Ribes,* and possibly other genera. The majority of ecosystems supporting conifer species across an entire continent have been invaded. The WPBR introductions were of such ecological importance that thousands of man-hours of research and control efforts were committed; these efforts continue today (Maloy 1997). WPBR introduction precipitated a major reduction in *Pinus monticola* Douglas ex. D. Don. (western white pine) populations in the northern Rocky Mountains (Neuenschwander *et al.* 1999). In this region, *P. monticola* was a modifier keystone species, whose presence influenced many ecosystem processes (McDonald *et al.* 2000). Removal of *P. monticola* has also revealed significant interactions between Armillaria root rot on replacement species and fire regimes that could ultimately produce shortened fire-return intervals and more intense stand-replacement fires (McDonald *et al.* 2003). Ecosystems where *P. albicaulis* Engelmann (whitebark pine) is a keystone species are also at risk to experience major perturbations (Tomback *et al.* 2001). The ecological importance of North American species of five-needle pines prompted much genetic and ecological investigation of the pathosystem, and a long-term extensive program of blister rust control was initiated (Maloy 1997).

Investigations were centered on three important commercial species *P. monticola, P. lambertiana* Douglas (sugar pine), and *P. strobus* L. (eastern white pine). A sketchy picture of blister rust genetics and biology in North America has emerged from much breeding for resistance and investigation of resistance gene / virulence factor couplets. An initial attempt at connecting this body of work to the ideas of evolutionary biology and phenotypic plasticity has been completed (McDonald *et al.* 2004). This summary shows that interactions are complex and changing. Other recent work shows the clear existence of durable resistance in western white pine (McDonald and Dekker-Robertson 1998) and existence of a typical R-gene / avirulence gene couplet in sugar, western white, and limber pines (Kinloch and Dupper 2002). Both kinds of resistance genes are probably present in *Ribes* as well (McDonald 2000, McDonald and Andrews 1981).

Ecologically important five-needle pines whose ranges are currently being invaded by WPBR are *P. flexilis* James (limber pine) and *P. strobiformis* Engelmann (southwestern white pine). Blister rust has not yet been found in natural stands of *P. aristata* Engelmann (bristle cone pine), *P. balfouriana* Greville & Balfor. (foxtail pine ), and was only recently reported in *P. longaeva* D. K. Bailey (Great Basin bristlecone pine, Blodgett and Sullivan 2004).

We first ask the question whether WPBR has become naturalized after 100 years in North America. It is very evident that the first wave of the rust epidemic killed sufficient trees to prevent the normal silviculture of *P. strobus*. On introduction, the rust appeared to cause very high amounts of damage. In the state of New York, destruction of entire

stands was documented (Snell 1931). In one case, a stand expected to produce 70,000 board feet was converted into a hardwood stand containing 157 *P. strobus*. Pine losses were projected to be about 75% of the stems. An important consideration is the biological, versus economic damage inflicted on the pine hosts. Is the continued pressure from rust mortality sufficient to result in the elimination of the pine hosts as viable populations, or the pines' ecologic roles? To address these issues, we will examine some of the clues left from 100 years of history across the entire North American continent. When this history is viewed in context with the evolutionary biology literature, we can make predictions about the course of "naturalization" of WPBR.

How does one measure pathogen impact in biological terms? For production foresters looking at a developing stand, only two measures are of consequence – mortality and defect. If stand stocking density is reduced during a rotation, the forester has a serious problem and white pine becomes economically unattractive. Costs of defect, including rot in infected trees, are similar to those for mortality. However, if maintenance or restoration of ecologic processes is the principal goal, other factors come into play. Additionally, persistence of sub-optimal numbers of resistant pines in a "generational bridge" could tie what is available and needed for ecological processes back to needs of practical forestry in the next generation. If such ecological processes are the goal then other measures of rust behavior become important and evolutionary interactions among hosts and pathogens become critical. Behaviors, such as epidemic asymptote, shape of disease-progress curves, rates of disease increase, canker growth rates, infection efficiency, infection distributions, and genetic structures of populations, become important guideposts to understanding evolutionary interactions.

After 100 years, at a time when the introduced microbe occupies nearly all of the suitable habitats on the North American continent, a curious thing has happened. Throughout most of its range, *P. strobus* is still a viable component. After 70 years of exposure to the rust in Maine, Ostrofsky *et al.* (1988) examined 9065 *P. strobus* trees representing 90 reproduction-to-pole stands and found 6.4% infection and 4.2% mortality due to blister rust. In the Upper Peninsula of Michigan, a survey of ca 5000 *P. strobus* conducted in 1984 showed only 1.5% infection (Robbins *et al.* 1988). Such current assessments contrast dramatically with historical accounts of blister rust impact. The same pattern has been found in some areas of western North America. At a site near Garibaldi, BC, Canada, shortly after introduction of the pathogen, 11 years of exposure of sapling- and pole-sized trees caused 90% mortality, almost all of which was from the death of branches from large numbers of infections (Lachmund 1934). On a plot 300 m removed from the heavy concentration of *Ribes* on the aforementioned plot, infection seemed to plateau at about 90% (Lachmund 1934). Mortality in this stand was at 11% by 1931, but unlike the first site, was mostly caused by girdling of the main bole (Lachmund 1934). Because recent studies have typically focused on blister rust behavior in resistant plantings, little data about the behavior of the rust in wild stands is currently available. However, an inspection of three of the original 1923 introduction centers in north Idaho (see Mielke 1943) showed relatively robust regeneration (McDonald, G. I. unpublished data). A thorough inspection of the 16 original introduction sites is needed to assess changes in blister rust behavior and impacts.

**Overdispersion in WPBR.** Fraker (1936) determined that, based on WPBR S-I (severity / incidence) curves, infections were not randomly distributed. The actual number of cankers per infected tree, given the number of total infections in the stand, was always larger than the expected number, demonstrating overdispersion. He devised an index for observed deviation from expected infections per tree and attributed the overdispersion to distribution of *Ribes*.

Here we examine overdispersion from S-I curves developed from published data for the following white pine species: 1) *P. monticola* in coastal British Columbia (anonymous 1923), see figure 5.6; 2) *P. monticola* size class exposed from 1917 to 1937 in interior British Columbia (Buchanan and Kimmey 1938) ($r^2 = .98$ K = 0.78 * 0.04; not shown); 3) *P. strobiformis* growing on plots located in the Sacramento Mountains of New Mexico and exposed from 1985 to 1997 (van Arsdel *et al.* 1998) ($r^2 = .98$ K = 0.97 * 0.04; not shown); and 4) *P. lambertiana* growing on plots in the central Sierra Mountains of California and exposed from 1970 to 1980 (Kliejunas 1982) ($r^2 = .99$ K = 0.78 * 0.03; not shown). Overdispersion was also shown by a rust-progress curve developed from aging cankers on *P. strobus* growing in Maine (Fracker 1936) (See Figure 5.7). The canker-aging technique is also suitable for development of S-I curves and was used to assess blister rust on western white pine growing in northern Idaho for exposure from 1925 to 1967 (McDonald unpublished data) ($r^2 = .99$ K = 0.92 * 0.008; not shown).

All of these situations show significant overdispersion, with asymptotes ranging from 0.78 to 0.97 during the original expansion of blister rust in North America. Why is overdispersion such a common feature associated with the introduction of blister rust? As discussed above, genetic interaction is the most common cause of this behavior in most pest systems. Such findings in WPBR should be taken as preliminary evidence of a developing resistance structure in five-needle pine populations. A recent analysis of long-term results generated by the breeding of a blister rust-resistant, *P. monticola* population indicates that native populations of the northern Rocky Mountains may have been capable of a rapid accumulation of resistance (McDonald *et al.* 2004).

The life histories of the hosts and pathogen involved in WPBR interactions can be examined through the perspective of evolutionary epidemiology. The applications of general theories of host-pest interaction can increase analytical power and create fresh perspectives, including landscape perspectives, of this important disease. Life histories of most WPBR pine infections have predictable patterns. Generally, a single haploid infection produces an

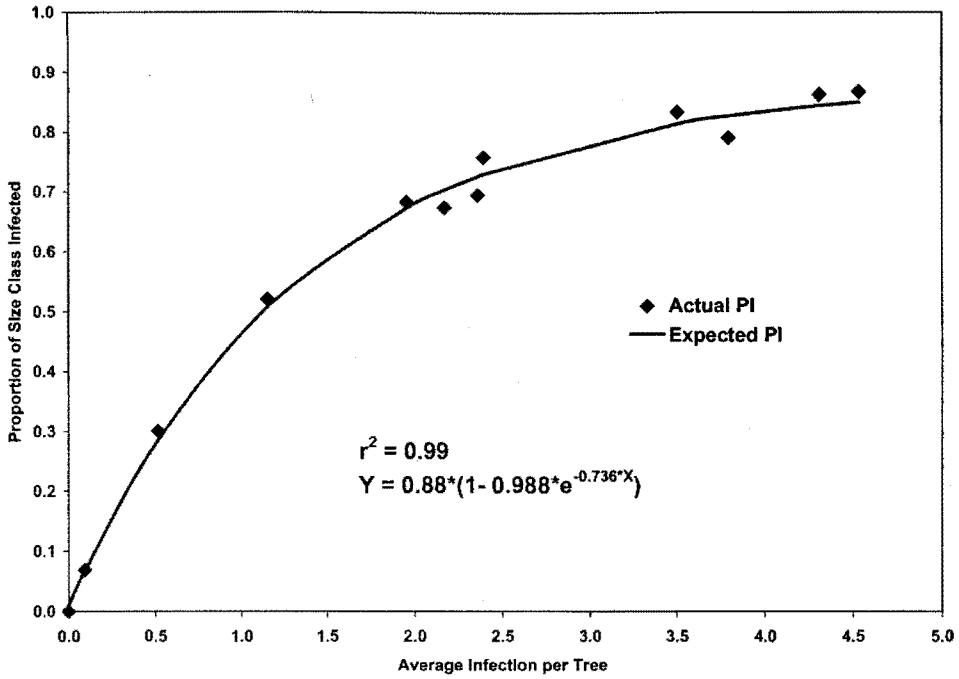


**Figure 5.6.** Severity-incidence curve on western white pine exposed to blister rust from 1913 to 1925 near Vancouver, BC at Brackendale (Anonymous 1923). Data were collected by tree-size classes receiving equal exposure time. Curve was fit using Madden and Campbell (1990) monomolecular equation and Table Curve software. The asymptote (maximum infection) is 0.88 ± 0.02.

elongated swelling on a branch or small bole. This individual will then produce flexuous (receptive) hyphae and pycniospores (spermatia) of either + or – mating type until the pycnium (spermagonium) is fertilized by a pycniospore of the opposite mating type. Fertilization generally occurs during the summer months, and aeciospores erupt the next spring from areas of the bark that previously supported pycnia. The amount of bark disruption is associated with the amount of aeciospore production, and is also related to the amount of host phloem that will be killed. When the combination of canker growth and bark disruption from aeciospore production girdles a branch or stem, the infection is at risk of dying. WPBR is a biotroph and relies on nutrients from the living cells. Severely damaged, girdled branches will often desiccate and die back to the next living node. Thus, the infection will die when it fails to bridge from one whorl supporting living branches to the next. The fungus may facilitate this bridge by supplying compounds to keep the internode bark alive (Lachmund and Hansbrough 1932). At a branch level, host life-history traits (e.g., years of needle retention, growth form, and growth rate) will influence the probability of death for the individual haploid strain that produced the infection. If a rust strain that grows rapidly and produces many aeciospores infects a host having short-duration needle retention and fast growth (long internodes), a single crop of aeciospores may be expected. Alternatively, if slow-growing and low-spore-producing rust infects a host with long-duration needle retention and slow growth (short internodes), many years of low to moderate levels of spore production may ensue. Thus, the WPBR pathosystem appears to harbor opportunities for life-history trade offs that influence the timing of death of diseased tissues. The evolutionarily stable strategy for the rust may be embodied in strains that generate moderate to large lifetime production of new infections. A similar rationale holds for whole trees. If a fast-growing, prolific strain succeeded in reaching the bole of a fast-growing, short-needle-retention host, it would kill the host and all of its infections. However, if this infection occurred on a slow-growing, multi-stemmed host, only a portion of the infections would be removed from the rust gene pool when one of these main stems is killed. Environment and host resistance genes would all interact toward achieving evolutionary stability.

A possibly significant life history variable that exists in North American WPBR populations is a dichotomy between slow- and fast-growing cankers. Early observations indicated that cankers on *P. strobus* sometimes died quickly, causing a collapse of the infection (Spaulding 1911). Other cankers on *P. strobus* grew more in length than in width, delaying stem girdling. Most cankers grew rapidly; some were slow to start and then grew at normal rates; while others started at normal rates then slowed dramatically or stopped (Rhodes 1920). More importantly, some cankers grew slowly and produced few aecia (Rhodes 1920). Phelps and Weber's (1969) description of canker types and mortality rates of cankers in *P. strobus* recognized three types: 1) "text book" fast growing and fast girdling, 2) slow growing with no sporulation, and 3) grooves and no sporulation. Regarding canker mortality in 35- to 45-year-old stands in the open, 45% of the cankers died in 5 years; whereas 70% of the cankers died in 5 years within closed stands. These authors also observed that production of aecia was high on young cankers and sparse on older cankers, but there was no indication whether the older cankers had been high or low aecia producers at an earlier age. Also, a much higher

proportion of cankers on *P. strobus* produced pycnia than produced aecia.

As with *P. strobus*, Mielke (1943) noted that some cankers on *P. monticola* never produced aecia. For the WPBR epidemic on *P. monticola*, canker "inactivation" was also studied. Activation state (visibility of mycelia margin) and age (year of supporting wood – 1 yr) were recorded on about 2000 cankers located on 48 plots in 1965 (northern Rockies). We used published data (Kimmey 1969) to fit age vs. proportion of cankers inactivated to a standard monomolecular equation (inactivation prop = $a(1- be^{-cx})$). In the case of cankers summed across all plots, with age = 1 at y = 0 (no inactivation at age 1), the asymptote (y = 1; all inactive) was reached at 30 years and the inactivation rate (mortality rate) was 0.08/year, with an $r^2 = 0.96$. However, the 48 individual plots could be divided into two kinds of plots by a curve-fitting procedure based on ages and rates of inactivation. The equation was fit to all data and outliers (> 2 s.d.) were removed. After three iterations, the population of plots could be readily divided into two groups: high mortality ($r^2 = 0.94$, c = 0.2, y intercept = 4, a = 0.9) and low mortality ($r^2 = 0.77$, c = 0.6, y intercept = 8, a = 0.6)

Several explanations of the above patterns of growth, mortality, and spore production can be suggested. One explanation is that two kinds of rust were present in the original introduction. We hypothesize that some local populations of WPBR underwent a rapid adjustment in their aggressiveness. This adjustment could represent progress toward an evolutionarily stable life history strategy that is an integral part of the WPBR pathosystem that arose after some 140 million years of coevolution with five-needle pines at diverse locations (McDonald *et al.* in preparation). Other considerations are whether life history traits of the pathogen or of individual cankers could be interacting with secondary fungi (Hunt 1997, Kimmey 1969, Wicker and Yokata 1982) that suppress sporulation and/or contribute to canker mortality. Other issues are the influence of host-life histories, such as growth rate, growth patterns, needle retention times, and host resistance. Alternate hosts may also be involved in this process. For example, some infections of *Ribes* hosts by aeciospores were observed in India (Bagshee 1950) and North America (McDonald and Andrews 1980) to produce only telia, which may indicate evolution or phenotypic plasticity. These observations could also be related to the species of alternate host, since Meilke (1943) observed that aecial infections formed telia directly on *R. lacustre*, but produced five consecutive generations of uredinia on nearby *R. viscosissimun*. These aspects warrant a more detailed investigation.

**Local structure in WPBR in Asia and North America.** In Asia, the WPBR pathosystem has coevolved more or less continuously for approximately 100 million years (McDonald *et al.* in preparation). As a result, geographical structure now exists for pine hosts, alternate hosts, and life cycles in four definable situations: central China; Korea and Japan; India; and northeastern Siberia and the Kamchatka Peninsula. The diversity of interactions among the rusts that occur on the various hosts and alternate hosts in each of the four regions and specific differences in virulence, breadth of host range, and age of host materials affected are of critical interest toward inferring the probable origins and outcomes of coevolution of blister rust in North America. Typically, the pathosystem causes little damage to pine hosts in Asia. Exceptions are plantations of *P. koraiensis* (Korean stone pine) in Korea, introduced *P. strobus* in Japan, and, significantly, native stands of *P. pumila* (Japanese stone pine) in eastern Siberia and the Kamchatka Peninsula (Azbukina 1995, Kakishima *et al.* 1995).

**Blister rust in eastern Siberia.** Eastern Siberia has two endemic five-needle pine species, *P. pumila* and *P. koraiensis*, and potentially two blister rusts, *C. ribicola* and *C. kamtchaticum*. Alternate hosts for *C. ribicola* are *Grossularia reclinata* and 11 *Ribes* spp. The second rust, *C. kamtchaticum*, is believed to alternate to *Castilleja pallida* and three *Pedicularis* spp. In a survey for WPBR at Sikhote-Alin Reserve northeast of Vladivostok, Kakishima *et al.* (1995) found *P. koraiensis* to be common in the valley bottoms and mid-slope areas, although WPBR was rare. Similarly, no WPBR was found on the mountaintop *P. pumila*. Uredinia and telia were common on both *Ribes* and *Pedicularis*. The identification of infections on *Pedicularis* was questioned because *C. flaccidum*, on *P. sylvestris*, alternates to *Pedicularis* and the two rusts are indistinguishable on this alternate host. On the Ussuri Reserve near Vladivostok and the Bolschechtsirsky Reserve about 100 miles north of Vladivostok, Kakishima *et al.* (1995) could not find WPBR on common *P. koraiensis*, but did find it on *Ribes*. Additionally, a blister rust, *C. ribicola* and/or *C. flaccidum*, was found on *Pedicularis*. In contrast, Kakishima *et al.* (1995) found heavy infection on both *P. pumila* and *Ribes* spp. at reserves in the Magadan Region on the Siberian coast west of the Kamchatka Peninsula, whereas microcyclic forms of *C. ribicola* were not found, and no infection could be found on *Castilleja* or *Pedicularis* spp. According to Azbukina (1995), *C. kamtchaticum* can occur as serious outbreaks on *P. pumila* on the Kamchatka Peninsula and the neighboring Magadan region. Infection can attain 100% and heavy mortality was observed on occasion (Azbukina 1995) and the alternate host was reported to be *Castilleja* and *Pedicularis* spp.

**WPBR in China.** In northwestern China, two five-needle pines occur, *P. armandii* and *P. siberica* (Jing *et al.* 1995). On *P. armandii,* only a microcylic blister rust is found that can be serious, but it is restricted to trees under 35 years of age (Jing *et al.* 1995). WPBR alternating to *Ribes* from *P. siberica* is known in northwestern China (Jing *et al.* 1995). No information about incidence, mortality, or etiology was found for either pine host.

**WPBR in Japan.** Japan has five native five-needle pines, *P. pumila*, *P. parviflora*, *P. hakkodensis* (a natural *P. pumila* x *P. parviflora* hybrid), *P. armandii*, and *P. koraiensis*. Only *P. pumila* has native rust (Imazu and Kakishima 1995). The first report of *C. ribicola* was on *Ribes* in 1906 (Wicker and Yokota 1976). The known rust species are *C. ribicola*,

*Endocronartium yamabense*, *E. sahoanum* var. *sahoanum*, and *E. sahoanum* var. *hokkaidoense*. Planted *P. strobus* (1972) and *P. koraiensis* (1970's) experienced serious damage caused by a rust (Imazu and Kakishima 1995) that alternated to both *Ribes* and *Pedicularis* (Yokota and Hama 1981); however, artificial inoculation of *P. strobus* and *P. koraiensis* reproduced disease only on *P. strobus* (Yokota *et al.* 1975). Natural infections were found on *P. pumila* and *Pedicularis* species (Yokota *et al.* 1975). In central Honshu, WPBR was found only on *P. pumila* and *Pedicularis* spp., while local *Ribes* spp. and *P. parviflora* were uninfected (Imazu and Kakishima 1995). In artificial inoculations, aeciospores from *P. strobus* growing on Hokkaido and *P. pumila* growing on Mt. Tateyama infected both *Ribes* and *Pedicularis*, but aecia collected from *P. strobus* growing on Ruben Island infected only *Ribes* spp. (Yotota and Uozumi 1976).

Regarding the endo forms, some interesting associations emerge. One form, *E. yamabense,* is restricted to young twigs of *P. pumila* growing on wind-exposed summits and ridges. The other endo form, *E. sahoanum*, is found in two varieties. Variety *sahoanum* is found on young and old twigs of *P. pumila* at higher elevations in northern Honshu (Imazu and Kakishima 1995). Artificial inoculation of *P. pumila* has been attained by wounding or by synchronizing shoot development with spore release by placing seedlings in cold storage (Kaneko and Harada 1995). Variety *hokkaidoense* is found on young and old twigs of *P. pumila* at low elevation in special volcanic situations (Imazu and Kakishima 1995). Endo forms occur only in the absence of alternate hosts, and pathogenicity to other five-needle pines is unknown for all the endo forms. Also, the forms are morphologically distinct from each other and from *C. ribicola* (Imazu and Kakishima 1995).

**WPBR in Korea.** The rust was first reported in Korea on *P. koraiensis* in 1937 (Hyun and Koo 1981). In 1963, an outbreak started in young plantations and has caused serious problems since then (Hyun and Koo 1981). Korea has small populations of native *P. parviflora*, *P. pumila*, and *P. koraiensis* and plantations of *P. strobus*, all of which remain free of WPBR (La and Yi 1995). Large outbreaks in presumably planted stands started in 1972 and many thousands of infected trees were removed (La and Yi 1995). In one inoculation test of *P. koraiensis*, *P. strobus*, and *P. parviflora*, infection rates were 0.23, 0.41, and 0.0, respectively (La and Yi 1995). Yet, *P. strobus* has remained free from natural infection. The Korean rust infects both *Ribes* and *Pedicularis* in inoculation tests, but only *Pedicularis* is found carrying the rust in nature (La and Yi 1995). Rust variation regarding alternate host was summarized into four types that occur in various regions, maybe even relatively small locales. These are *Ribes-Pedicularis*, *Ribes*, *Pedicularis*, and *Castilleja* (Stephan and Hyun 1983). The *Castilleja* type is known only from artificial inoculations in Canada (Hiratsuka and Maruyama 1976).

**WPBR in India.** Bagshee (1950) collected aeciospores, which typically appear right after snow melt in early April, from Kashmir on June 20, 1935 (late spring). These aeciospores produced a short generation of uredinia when inoculated onto *R. rubrum*, then produced telia in 6 weeks. In India, a period of spring rains generally ends in early June. The monsoon, a continuous wet period, lasts from mid-July to early October. Needle retention on *P. wallichiana* varies from 1 year 3 months to 3 years. Some of

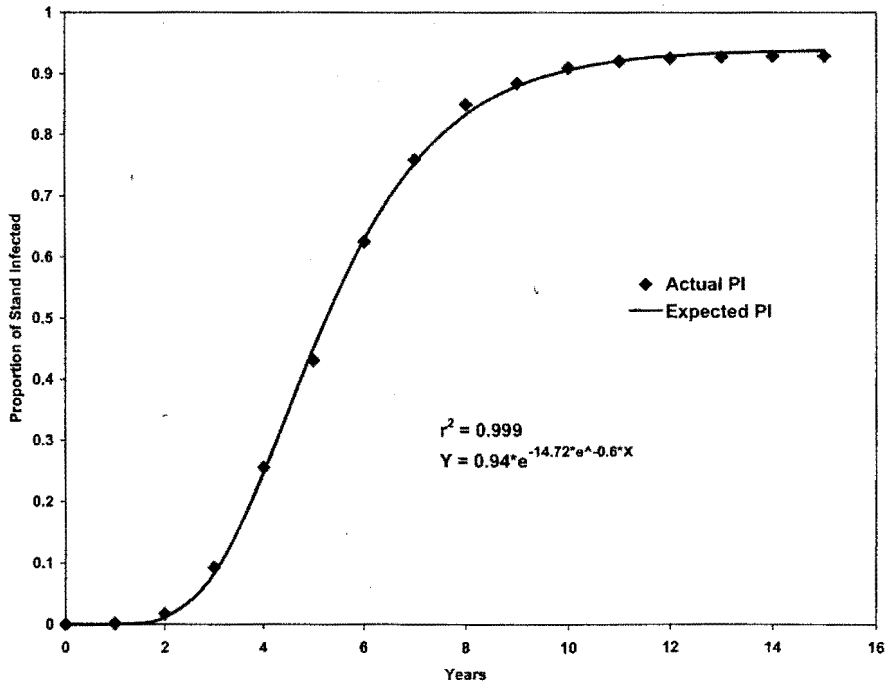


**Figure 5.7.** Rust-progress curve on eastern white pine exposed to blister rust from ca 1905 to 1923 in central Maine (Fracker 1936). Data were collected by counting and aging cankers on saplings growing in one stand. Curve was fit as in Figure 6 using the Gomperitz equation (Madden and Campbell 1990). The asymptote (maximum infection) is 0.94 ± 0.004.

Bagshee's figures (1950) appear to show differences in growth rates, but no data were recorded.

**Pine Cross-inoculation Experiments.** Aside from analysis of phylogenies and divergence times, older tools, such as geographic cross inoculations and careful observation of rust behavior in natural situations, provide insight into evolution of rust pathosystems. Most of the species of section *Strobus* have been artificially inoculated with western North American WPBR (Hoff *et al.* 1980). Pine responses were classified by incidence of needle infection (proportion of sampled seedlings), frequency of needle infections (spots per meter of needle length), incidence of needle clearing (proportion of spotted seedlings free of cankers 2 years after inoculation), and incidence of canker clearing (proportion of stem-infected seedlings with fully resistant bark reactions 3 years after inoculation). Although genetic interpretations were limited by inconsistency of seed-collection parameters, some general trends have emerged. Spot frequency was lowest in subsection *Cembrae* and the Asian representatives of subsection *Strobi* (average = 2 spots/m). Seedlings from a single phenotypically resistant *P. albicaulis* averaged about 7 spots/m. The North American subsection *Strobi* pines varied from a high of 28 spots/m for *P. ayacahuite* to a low of 4 spots/m for *P. lambertiana*. Putative basal Subsection *Balfourianae* averaged 61 spots/m. Besides fewer spots, Asian *Strobi* pines showed low to moderate incidence of infection and low to high incidence of both needle and stem clearing. In contrast, *P. morrisonicola*, a tropical pine, showed high incidence of infection, no needle clearing, and moderate stem clearing. Asian *P. wallichiana* inoculated in North America showed moderate infection incidence and moderate levels of needle and stem clearing. However, artificial inoculation of different samples of this pine in India with native rust showed low infection incidence, a high level of needle clearing, and a low level of stem clearing (Bagshee 1950). Subsection *Cembrae* gave low infection incidence with the exception of *P. albicaulis*, which gave moderate incidence. All the stone pines showed high levels of needle clearing and moderate levels of stem clearing. Field run North American *Strobi* pines gave high but variable infection incidence and low but variable levels of both stem and needle clearing, but responses of *P. monticola* selected for resistance resembled those of *P. wallichiana*. Unfortunately, similar data regarding *P. strobus* are unavailable. However, the sum of the evidence about WPBR indicates that the *P. strobus* gene pool coevolved with WPBR for about 100 million years and that a separation is likely less than 3 million years old. Evidence of the long-term ancient interaction coupled with evidence of a highly responsive resistance structure in extant pines indicates that functional remnants of coevolution undoubtedly remain in *P. strobus*. Management for natural *P. strobus* regeneration has a place in the effort to reconstitute the ancient host-pathogen structure.

# An Evolutionary Epidemiological Hypothesis for Blister Rust in North America

The behavior of WPBR on native *P. pumila* in Siberia argues for the existence of an evolutionarily stable strategy for high-spore production in a cold, short-season environment on a slow-growing, many-stemmed host. In this situation, the rust could likely sustain a high rate of spore production without killing all of the stems of its host. A rust like this could have exploded across Europe and come to North America. Such rust might not survive well on fast-growing hosts, unless it could reach the main stem. Even on the main stem, its aggressive behavior could lead to its early demise, because its host has only a single stem. Alternatively, infections that produce small amounts of pycniospores and aeciospores every year might live a long time and produce spores for many years. If long survivability were being selected for, a change in gene frequencies could happen rapidly on a local scale, because the nuclear condition of the rust on pine is haploid. Selective survival could occur by other means as well. Cankers growing in cold environments could be more like the Siberian situation where fast growth and high spore production have been maintained in the population. Nevertheless, fast-growing, high-spore-producing strains occurring on young seedlings in a warm environment would quickly be eliminated at the same time that the disease pressure is simultaneously selecting for resistant hosts and slow growing, low-spore-production stains. Strategically located infections would quickly kill their hosts, causing the loss of both host and pathogen from their respective populations. Meanwhile, hosts that could slow the rate of canker growth or that were infected by a slow-growing, low-spore-production strain would survive and continue as a source of spores.

Testing of this hypothesis will require the thorough study of all aspects of evolutionary epidemiology (overdispersion, evolution of virulence, local population structure, phenotypic plasticity or gene expression, consideration of introductions in an experimental context, and liberal use of properly designed geographic cross-inoculation experiments). Each of the four hypothesized Asian populations of blister rust as well as introduced populations in North America that have had ca 100 years to naturalize should be included in these studies. Of particular importance are an understanding of the relative roles of R-genes and r-genes in both *Ribes* and pine hosts. On the pathogen side, an understanding of the mechanics of evolution of virulence and the role of life cycle and other polyphenisms is needed. Key questions are the antiquity of the stem rust pathosystem and the degree to which the naturalization process has reestablished historic norms. Further examination of these features of WPBR would have significant impact on management and restorations efforts for North American five-needle pine and should help frame future discussions about the

evolutionary epidemiology of plant diseases. Widespread application of these ideas might also help plant disease epidemiologists become better disease detectives.

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## References

Agrawal, A. A., C. Laforsch, and R. Tollrian. 1999. Transgenerational induction of defences in animals and plants. Nature 401:60-63.

Andrews, J. H. 1991. Comparative Ecology of Microorganisms and Macroorganisms. Brock/Springer Series in Contemporary Bioscience. Springer-Verlag, New York. 302 p.

Andrews, J. H., and R. F. Harris. 2000. The ecology and biogeography of microorganisms on plant surfaces. Annu. Rev. Phytopathol. 28:145-180.

Anonymous. 1923. Relation of crown class to white pine infection on Brackendale area. Table 9. Proportion of dominant, intermediate, and overtopped trees in stand. Pp. 17 (18)-29 in: Blister rust work in the far West, February 1 to December 31, 1923. USDA Bureau of Entomology and Plant Quantine, Office of Blister Rust Control, Spokane, WA.

Azbukina, Z. M. 1995. *Cronartium* species on *Pinus* species in the Russian Far East. Pp. 65-69 in: S. Kaneko, K. Katsuya, M. Kakishima, and Y. Ono, eds. Proceedings of the Fourth IUFRO Rusts of Pines Working Party Conference, Tsukuba. Tsukuba, Japan.

Bagshee, K. D. 1950. Contributions to our knowledge of the morphology, cytology and biology of Indian coniferous rusts. Part II. Observations on the occurrence of *Cronartium ribicola* Fisher and *Peridermium indicum* Colley and Taylor on *Pinus excelsa* Wall. in India with reference to their distribution, pathology, inoculation experiments and comparative morphology. Indian Forest Records 4:1-41.

Baldwin, I. J. 2001. An ecologically motivated analysis of plant-herbivore interactions in native tobacco. Plant physiology 127:449-1458.

Bergelson, J., G. Dwyer, and J. J. Emerson. 2001. Models and data on plant-enemy coevolution. Annu. Rev. Genet. 35: 469-499.

Bird, A. 2002. DNA methylation patterns and epigenetic memory. Genes & Development 16:6-21.

Blodgett, J. T., K. F. Sullivan. 2004. First report of white pine blister rust on Rocky Mountain bristlecone pine. Plant Dis. 88:311.

Browning, J. A. 1974. Relevance of knowledge about natural ecosystems to development of pest management programs for agro-ecosystems. Pp. 191-199 in: Proceedings of the American Phytopathology Society, Vancouver, British Columbia, Canada.

Buchanan, T. S., and J. W. Kimmey. 1938. Initial tests of the distance of spread to and intensity of infection on *Pinus monticola* by *Cronartium ribicola* from *Ribes lacustre* and *R. viscosissimum*. Journal of Agricultural Research 56:9-30.

Burdon, J. J., and P. H. Thrall. 1999. Spatial and temporal patterns in coevolving plant and pathogen associations. The American Naturalist. 153:S15-S33.

Chandler, V. L., and H. Vaucheret. 2001. Gene activiation and gene silencing. Plant Physiology. 125:145-148.

Crespi, B. J. 2000. The evolution of maladaptation. Heredity. 84:623-629.

Day, T. 2003. Virulence evolution and the timing of disease life-history events. Trends in Ecology and Evolution. 18:113-118.

Dinoor, A., and N. Eshed. 1984. The role and importance of pathogens in natural plant communities. Annu. Rev. of Phytopathol. 22:443-466.

Ebert, D. 1998. Experimental evolution of parasites. Science. 282:1432-1435.

Ebert, D., and J. J. Bull. 2003. Challenging the trade-off model for the evolution of virulence: is virulence management feasible? Trends in Microbiology 11:15-20.

Ebert , D., and W. D. Hamilton. 1996. Sex against virulence: the coevolution of parasitic diseases. TREE 11:79-82.

Evans, J. D. and D. E. Wheeler. 1999. Differential gene expression between developing queens and workers in the honey bee, *Apis mellifera*. Proc. Natl. Acad. Sci. USA 96:5575-5580.

Evans, J. D., and D. E. Wheeler. 2001. Gene expression and the evolution of insect polyphenisms. BioEssays 23:62-68.

Ewald, P. W. 1994. Evolution of infectious disease. Oxford University Press, New York.

Ewald, P. W., J. B. Sussman, M. T. Distler, C. Libel, W. P. Chammas, V. J. Dirta, C. A. Salles, A. C Vincente, I. Hietmann, and F. Cabello. 1998. Evolutionary control of infectious disease: Prospects for vectorborne and waterborne pathogens. Mem. Inst. Oswaldo Cruz, Rio de Janeiro. 93:567-576.

Fenton, A., R. Wall, N. P. French. 1999. The effects of oviposition aggregation on the incidence of sheep blowfly strike. Veterinary Parasitology 83:137-150.

Flather, C. H., M. S. Knowles, I. A. Kendall. 1998. Threatened and endangered species geography. BioScience 48:365-376.

Frank, S. A. 2000. Specific and non-specific defense against parasitic attack. Jour. of Theor. Biol. 202:283-304.

Fracker, S. B. 1936. Progressive intensification of uncontrolled plant-disease outbreaks. Jour of Econ. Entomology 29: 923-940.

Galvani, A. P. 2003. Epidemiology meets evolutionary ecology. Trends in Ecology and Evolution. 18:132-139.

Gandon, S., and P. A. Van Zandt. 1998. Local adaptation and host-parasite interactions. TREE 13:214-216.

Gilbert, G. S. 2002. Evolutionary ecology of plant diseases in natural ecosystems. Annu. Rev. Phytopathol. 40:13-43.

Hanski, I., and M. E. Glipin. 1997. Metapopulation biology: ecology, genetics, and evolution. Academic Press, San Diego, CA. Pg. 512.

Heath, M. C. 1991. Tansley review no. 33 evolution of resistance to fungal parasitism in natural ecosystems. New Phytol. 119:331-343.

Hoff, R. J., R. T. Bingham, and G. I. McDonald. 1980. Relative blister rust resistance of white pines. Eur. J. For. Path. 10: 307-316.

Hiratsuka, Y., and P. J. Maruyama. 1976. *Castilleja miniata*, a new alternate host of *Cronartium ribicola*. Plant Disease Reporter 60:241.

Hunt, R. S. 1997. Relative value of slow-canker growth and bark reactions as resistance responses to white pine blister rust. Canadian Journal of Plant Pathology 19:352-357.

Hyun, S. K., and Y. B. Koo. 1981. Possibility of breeding of blister rust resistant synthetic clones of Korean pine (*Pinus koraiensis* S. et Z.). pp. 239-245 in: Proceedings of the 17th IUFRO World Congress. Division 2, Forest plants and forest protection. Japanese IUFRO Congress Committee. Ibaraki, Japan.

Imazu, M., and M. Kakishima. 1995. The blister rusts on *Pinus pumila* in Japan. Pp. 27-36 in: S. Kaneko, K. Katsuya, M. Kakishima, and Y. Ono, eds. Proceedings of the fourth IUFRO Rusts of Pines working party conference, Tsukuba. Forestry and Forest Products Research Institute. Ibaraki, Japan.

Janeway, C. A., Jr., and R. Medzhitov. 2002. Innate immune recognition. Annual Rev. Immunol. 20:197-216.

Jaenisch, R., and A. Bird. 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nature genetics supplement 33: 245-254.

Jarosz, A. M., and A. L. Davelos. 1995. Effects of disease in wild plant populations and the evolution of pathogen aggressiveness. New. Phytol. 129:371-387.

Jing, Y., W.-H. Li, and SW.-G. Zhao. 1995. Study on pine rusts in Northwest China. Pp. 37-41 in: S. Kaneko, K. Katsuya, M. Kakishima, and Y. Ono, eds. Proceedings of the fourth IUFRO Rusts of Pines working party conference, Tsukuba. Forestry and Forest Products Research Institute. Ibaraki, Japan.

Kakishima, M., M. Imazu, K. Katsuya, Z. M. Azbukina, Y. Ono, S. Kaneko, Y. Hiratsuka, and S. Sato.1995. Preliminary survey of pine blister rusts in the Russian Far East. Pp. 49-63 in: S. Kaneko, K. Katsuya, M. Kakishima, and Y. Ono, eds. Proceedings of the fourth IUFRO Rusts of Pines working party conference, Tsukuba. Forestry and Forest Products Research Institute. Ibaraki, Japan.

Kaltz, O., and J. A. Shykoff. 1998. Local adaptation in host-parasite systems. Heredity. 81:361-370.

Kaneko, S., and Y. Harada. 1995. Life cycle of *Endocronartium sahoanum* and its nuclear condition in axenic culture. Pp. 95-100 in: S. Kaneko, K. Katsuya, M. Kakishima, and Y. Ono, eds. Proceedings of the fourth IUFRO Rusts of Pines working party conference, Tsukuba. Forestry and Forest Products Research Institute. Ibaraki, Japan.

Kinkel, L. L. 1997. Microbial population dynamics on leaves. Annu. Rev. Phytopathol. 35:327-347.

Kim, Y.-K., and W. W. Bockus. 2003. Temperature-sensitive reaction of winter wheat cultivar AGSECO 7853 to *Stagonospora nodorum*. Plant Disease 87:1125-1128.

Kimmey, J. W. 1969. Inactivation of lethal-type blister rust cankers on western white pine. Journal of Forestry. 67: 296-299.

Kimmey, J. W., and J. L. Mielke. 1944. Susceptibility to white pine blister rust of *Ribes cereum* and some other *Ribes* associated with sugar pine in California. Journal of Forestry 42:752-756.

Kinloch, B. B., Jr., and G. E. Dupper. 2002. Genetic specificity in the white pine-blister rust pathosystem. Phytopathology 92:278-280.

Kirchner, J. W., and B. A. Roy. 1999. The evolutionary advantages of dying young: Epidemiological implications of longevity in metapopulations. American Naturalist 154:140-159.

Kirchner, J. W., and B. A. Roy. 2001. Evolutionary implications of host-pathogen specificity: the fitness consequences of host life history traits. Evolutionary Ecology 14:665-692.

Kirchner, J. W., and B. A. Roy. 2002. Evolutionary implications of host-pathogen specificity: the fitness consequences of pathogen virulence traits. Evolutionary Ecology Research 4:27-48.

Kliejunas, J. 1982. A biological evaluation of white pine blister rust on the Sierra National Forest. Report No. 82-44. U.S. Dept. of Agriculture, Forest Service, Pacific Southwest Region, State and Private Forestry, Forest Pest Management, San Francisco, CA. 19 p.

Kloppers, F. J., and Z. A. Pretorius. 1997. Effects of combinations amongst Lr13, Lr34 and Lr37 on components of resistance in wheat to leaf rust. Plant Pathology 46:737-750.

Kolmer, J. A. 1996. Genetics of resistance to wheat leaf rust. Annu. Rev. Phytopathol. 34:435-455.

La, Y.-J., and C.-K. Yi. 1995. Occurrence and management of Korean pine blister rust in Korea. Pp. 171-175 in: S. Kaneko, K. Katsuya, M. Kakishima, and Y. Ono, eds. Proceedings of the fourth IUFRO Rusts of Pines working party conference, Tsukuba. Forestry and Forest Products Research Institute. Ibaraki, Japan.

Lachmund, H. G. 1934. Damage to *Pinus monticola* by *Cronartium ribicola* at Garibaldi, Bristish Columbia. Journal of Agricultural Research 49:239-249.

Lachmund, H. G., and J. R. Hansbrough. 1932. Preliminary report on the relative susceptibility of sugar pine and western white pine to blister rust. Journal of Forestry 30:687-691.

Line, R. F. 2002. Stripe rust of wheat and barley in North America: a retrospective historical review. Annu. Rev. Phytopathol. 40:75-118.

Lipsitch, M., and E. R. Moxon. 1997. Virulence and transmissibility of pathogens: what is the relationship? Trends in Microbiology 5:31-37.

Little, T. J. 2002. The evolutionary significance of parasitism: do parasite-driven genetic dynamics occur *ex silico*? J. Evol. Biol. 15:1-9.

Lorys, M. M. A., V. Lannou, and C. Lannou. 2000. Selection for increased spore efficacy by host genetic background in a wheat powdery mildew population. Phytopathology 90:1300-1306.

Lundquist, J. E., and N. B. Klopfenstein. 2001. Integrating concepts of landscape ecology with the molecular biology of forest pathogens. Forest Ecology and Management 150: 213-222.

Madden, L. V., and C. L. Campbell. 1990. Nonlinear disease progress curves. Pages 181-229 in: J. Kranz editor. Epidemics of plant disease: Mathematical analysis and modeling. Springer-Verlag, Berlin.

Maloy, O. C. 1997. White pine blister rust control in North America: A case history. Annu. Rev. Phytopathol. 35:87-109.

McDonald, B. A., and C. Linde. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annu. Rev. Phytopathol. 40:349-379.

McDonald, G. I. 1974. Introductory Remarks. Pg 170 in Proceeding of the American Phytopathology Society, Vancouver, British Columbia, Canada.

McDonald, G. I. 1996. Ecotypes of blister rust and management of sugar pine in California. Pp. 137-147 in: B. B. Kinloch, Jr., M. Marosy, and M. E. Huddleston, eds. Sugar pine. Status values, and roles in ecosystems. Publication 3362. University of California Division of Agriculture and Natural Resources. Davis, CA.

McDonald, G. I. 2000. Geographic variation of white pine blister rust aeciospore infection efficiency and incubation period. HorTechnology 10:533-536

McDonald, G. I., J. Evans, M. Moeur, T. Rice, and E. Strand, E. Using digital terrain models and satellite imagery to map interactions among fire and forest microbes. Pp. 100-110 in: K. E. M. Galley, R. C. Klinger, and N. G. Sugihara, eds. Proc. of Fire Conference 2000: The First National congress on Fire Ecology, Prevention, and Management. Misc. Publ. No. 13, Tall Timber Research Station, Tallahassee, FL.

McDonald, G. I., A. E. Harvey, and J. R. Tonn. 2000. Fire, competition and forest pests: landscape treatment to sustain ecosystem function. Pp. 195-211 in: L. F. Neuenschwander, and K. C. Ryan, technical eds. The joint fire science conference and workshop: proceedings; 1999 June 15-17; Boise, ID. Moscow, ID: University of Idaho and The International Association of Wildland Fire.

McDonald, G. I., and D. S. Andrews. 1980. Influence of temperature and spore stage on production of teliospores by single aeciospore lines of *Cronartium ribicola* conifer rust, grown on *Ribes hudsonianum* var. *petiolare*. USDA Forest

Service research paper INT - United States, Intermountain Forest and Range Experiment Station. Pp. 256-265.
McDonald, G. I., and D. S. Andrews. 1981. Genetic interaction of *Cronartium ribicola* and *Ribes hudsonianum* var. *petiolare*. Forest Science 27:758-763.
McDonald, G. I., and D. L. Dekker-Robertson. 1998. Long-term differential expression of blister rust resistance in western white pine. In: Proc. First IUFRO Rusts of Forest Trees WP Conf., 2-7 Aug., 1998, Saariselkä, Finland, Finnish Forest Research Institute, Research Papers 712: 285-295.
McDonald, G. I., and R. J. Hoff. 2001. Blister rust: An introduced plague. Chapter 10. Pp. 193-220 in: D. F. Tomback, S. F. Arno, and R. E. Keane, eds. Whitebark pine communities. Ecology and restoration. Island Press, Washington, DC.
McDonald, G. I., P. Zambino, and R. Sniezko. 2004. Breeding rust-resistant five-needle pines in western United States: Lessons from the past and a look to the future. Pp 28-50 in: R. A. Sniezko, S. Samman, S. E. Schlarbaum, and H. B. Kriebel, eds. Breeding and genetic resources of five-needle pines: growth adaptability, and pest resistance. IUFRO Working Party 2.02.15: proceedings; 2001 July24-25; Medford, OR, USA. Fort Collins, CO: U.S.D.A. Forest Service, Rocky Mountain Research Station. RMRS-P-32.
Meilke, J. L. 1943. White pine blister rust in western North America. Yale University School of Forestry Bulletin 52, New Haven, CT.
Milgroom, M. G. 2001. The synthesis of genetics and epidemiology: contributions of population biology in plant pathology. Journal of Plant Pathology 83:57-62.
Mopper, S., J. B. Mitton, T. G. Whitham, N. S. Cobb, and K. M. Christensen. 1991. Genetic differentiation and heterozygosity in pinyon pine associated with resistance to herbivory and environmental stress. Evolution 45:980-999.
Mopper, S. 1996. Adaptive genetic structure in phytophagous insect populations. TREE 11:235-238.
Moricca, S. and A. Ragazzi. 1998. Use of RFLP and SSCP analysis to differentiate the pine rusts. Mycological Research 102:666-670.
Neuenschwander, L. F., J. W. Byler, A. E. Harvey, G. I. McDonald, D. S. Ortiz, H. L. Osborne, G. C. Snyder, and A. Zack. 1999. White pine in the American west: A vanishing species—can we save it? USDA USFS General Technical Report RMRS-GTR-35: 20 p.
Nijhout, H. F. 1999a. Control mechanisms of polyphonic development in insect. BioScience 49:181-191.
Nijhout, H. F. 1999b. When developmental pathways diverge. Proc. Natl. Acad. Sci. USA 96:5348-5350.
Nijhout, H. F. 2003. Development and evolution of adaptive polyphenisms. Evolution and Development 5:9-18.
Ostrofsky, W. D., T. Rumpf, D. Struble, and R. Bradbury. 1988. Incidence of white pine blister rust in Maine after 70 years of a *Ribes* eradication program. Plant Disease 72:967-970.
Pigliucci, M. 2001. Phenotypic Plasticity: Beyond Nature and Nurture. The Johns Hopkins University Press. Baltimore, MD. 328.
Phelps, W. R., and R. Weber. 1967. Characteristics of blister rust cankers on eastern white pine. Research Note NC-80. U.S. Dept. of Agriculture, Forest Service, North Central Forest Experiment Station. St. Paul, MN.
Pollard, H., M. Cruzan, and M. Pigliucci. 2001. Comparative studies of reaction norms in *Arabidopsis*. I. Evolution of response to daylength. Evolutionary Ecology Research 3: 129-155.
Relyea, R. A. 2002. Costs of phenotypic plasticity. The American Naturalist 159:272-282.
Remold, S. K., and R. E. Lenski. 2001. Contribution of individual random mutations to genotype-by-environment interactions in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 98:11388-11393.
Rhodes, A. S. 1920. Studies on the rate of growth and behavior of the blister rust on white pine in 1918. Phytopathology 10: 513-527.
Robbins, K., W. A. Jackson, and R. E. McRoberts. 1988. White pine blister rust in the eastern upper peninsula of Michigan. North J. Appl. For. 5:263-264.
Roy, B. A., and J. W. Kirchner. 2000. Evolutionary dynamics of pathogen resistance and tolerance. Evolution 54:51-63.
Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson, and S. G. Weller. 2001. The population biology of invasive species. Annu. Rev. Ecol. Syst. 32:305-332.
Savile, D. B. O. 1976. Evolution of the rust fungi (Uredinales) as reflected by their ecological problems. Evolutionary Biology 9:137-206.
Schilichting, C. D., and M. Pigliucci. 1995. Gene regulation, quantitative genetics and the evolution of reaction norms. Evolutionary Ecology 9:154-168.
Schmitt, J. S., A. Dudley, and M. Pigliucci. 1999. Manipulative approaches to testing adaptive plasticity: Phytochrome-mediated shade-avoidance responses in plants. 154:S43-S54.
Seeley, R. H. 1986. Intense natural selection caused a rapid morphological transition in a living marine snail. Proc. Natl. Acad. Sci. USA 83:6897-6901.
Seem, R. C. 1984. Disease incidence and severity relationships. Ann. Rev. Phytopathol. 22:133-150.
Slev, P. R., and W. K. Potts. 2002. Disease consequences of pathogen adaptation. Current Opinion in Immunology 14: 609-614.
Snell, W. H. 1931. Forest damage and the white pine blister rust. Jour. of Forestry 29:68-78.
Sork, V. L., K. A. Stowe, and C. Hochwender. 1993. Evidence for local adaptation in closely adjacent subpopulations of northern red oak (*Quercus rubra* L.) expressed as resistance to leaf herbivores. Evolution 142:928-936.
Spaulding, P. C. 1911. The blister rust of white pine. USDA Bulletin Number 206, Washington, D.C.
Spaulding, P. C. 1922. Investigations of the white pine blister rust. USDA Bulletin Number 957, Washington, D.C.
Staskawicz, B. J. 2001. Common and contrasting themes of plant and animal diseases. Science 292:2285-2289.
Stephan, B. R. and S. K. Hyun. 1983. Studies on the specialization of *Cronartium ribicola* and its differentiation on the alternant hosts *Ribes* and *Pedicularis*. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 90:670-678.
Stokes, T. L., B. N. Kunkel, and E. J. Richards. 2002. Epigenetic variation in Arabidopsis disease resistance. Genes and Development 16:171-182.
Thompson, J. N. 1994. The coevolutionary process. Univ. of Chicago Press, Chicago, IL.
Thompson, J. N. 1997. Evaluating the dynamics of coevolution among geographically structured populations. Ecology 78:1619-1623.
Thompson, J. N. 1998. Rapid evolution as an ecological process. TREE 13:329-332.
Thompson, J. N. 1999a. Coevolution and escalation: Are ongoing coevolutionary meanderings important? American Naturalist. 153:S92-S93.
Thompson. J. N. 1999b. The evolution of species interactions. Science 284:2116-2118.
Thrall, P. H., and J. J. Burdon. 1997. Host-pathogen dynamics in a metapopulation context: the ecological and evolutionary consequences of being spatial. Journal of Ecology 85:743-753.
Thrall, P. H., and J. J. Burdon. 1999. The spatial scale of pathogen dispersal: Consequences for disease dynamics and persistence. Evolutionary Ecology Research 1:681-701.

Thrall, P. H. and J. J. Burdon. 2003. Evolution of virulence in a plant host-pathogen metapopulation. Science 299:1735-1737.

Thrall, P. H., J. J. Burdon, and A. Young. 2001. Variation in resistance and virulence among demes of a plant host-pathogen metapopulation. Journal of Ecology 89:736-748.

Tollrian, R., and C. D. Harvell. 1999. The ecology and evolution of inducible defenses. Princeton University Press, Princeton, NJ.

Tomback, D. F., S. F. Arno, and R. E. Keane. 2001. The compelling case for management intervention. Pp. 3-25 in: D. F. Tomback, S. F. Arno, and R. E. Keane, eds. Whitebark pine communities. Ecology and restoration. Island Press, Washington, DC.

Torchin, M. E., K. D. Lafferty, and A. M. Kuris. 2002. Parasites and marine invasions. Parasitology 124:S137-S151.

Trussell, G. C., and L. D. Smith. 2000. Induced defenses in responses to an invading crab predator: an explanation of historical and geographic phenotypic change. Proc. Natl. Acad. Sci. USA 97:2123-2127.

Tsutsui, N. D., A. V. Suarez, D. A. Holway, and T. J. Case. 2000. Reduced genetic variation and the success of an invasive species. Proc. Natl. Acad. Sci. USA 97:5948-5953.

Van Arsdel, E. P., D. A. Conklin, J. B. Popp, and B. W. Geils. 1998. The distribution of white pine blister rust in the Sacramento Mountains of New Mexico. In: Proc. First IUFRO Rusts of Forest Trees WP Conf., 2-7 Aug., 1998, Saariselkä, Finland, Finnish Forest Research Institute, Research Papers 712:275-283.

Van Baalen, M., and M. W. Sabelis. 1995. The dynamics of multiple infection and the evolution of virulence. American Naturalist 146:881-910.

Van Zandt, P. A., and S. Mopper. 1998. A meta-analysis of adaptive deme formation in phytophagous insect populations. American Naturalist 152:595-604.

Waggoner, P. E., and D. E. Aylor. 2000. Epidemiology: a science of patterns. Annu. Rev. Phytopathol. 38:71-94.

Waggoner, P. E., and S. Rich. 1981. Lesion distribution, multiple infection, and the logistic increase of plant disease. Proc. Natl. Acad. Sci. USA 78:3292-3295.

Waters, C. W. 1928. The control of teliospore and urediniospore formation by experimental methods. Phytopathology 18: 157-213.

Westerman, P. R. 1999. Aggregation of entomopathogenic nematodes, *Heterorhabditis* spp. and *Steinernema* spp., among host insects at 9 and 20°C and effects on efficacy. Journal of Invertebrate Pathology 73:203-213.

Wicker, E. F., and S.-I. Yokota. 1976. On the *Cronartium* stem rust(s) of five-needle pines in Japan. Ann. Phytopath. Soc. Japan 42:187-191.

Wicker, E. F., and S.-I. Yokota. 1982. Fungi associated with blister rust cankers on *Pinus strobus* and *P. pumila* in Japan. Trans. Mycol. Soc. Japan 23:143-148.

Wilcove, D. S., D. Rothstein, J. Dubow, A. Phillips, E. Losos. 1998. Quantifying threats to imperiled species in the United States. BioScience 48:607-615.

Wu, R. 1998. The detection of plasticity genes in heterogeneous environments. Evolution 52:967-977.

Yokota, S.-I., and T. Hama. 1981. On the race of *Cronartium ribicola*, the causal fungus of the blister rust of white pines (interim report). Pp. 230-239 in: Proceedings of the 17th IUFRO World Congress. Division 2, Forest plants and forest protection. Japanese IUFRO Congress Committee. Ibaraki, Japan.

Yokota, S.-I., T. Uozumi. 1976. New developments in white pine blister rusts in Japan. Pp. 330-342 in: Proceedings of the 16th IUFRO World Congress, Division II. Oslo, Norway.

Yokota, S., T. Uozumi, K. Endo, and S. Matsuzaki. 1975. A *Cronartium* rust of strobe pine in eastern Hokkaido, Japan. Plant Disease Reporter 59:419-422.

Zhan, J., C. C. Mundt, M. E. Hoffer, and B. A. McDonald. 2002. Local adaptation and effect of host genotype on the rate of pathogen evolution: an experimental test in a plant pathosystem. J. Evol. Biol. 15:634-647.