After 100 Years, Is Coevolution Relevant?

Geral I. McDonald[1]

> "This potential independence between DNA and epigenetic genotype for a gene even raises the potential for intragenic epistasis whereby the DNA and epigenetic genotypes for a gene interact to determine the phenotypic outcome. The simplest example would be where the DNA polymorphism toggles between a functional and nonfunctional allele and the epigenetic polymorphism toggles a silent and expressed gene." (p 483 Kliebenstein 2010)

On the 100th anniversary of the introduction of *Cronartium ribicola* into western North America, it is fitting to assess the philosophical foundation of plant pathology and forest ecology. We should ask whether this foundation provides sufficient understanding of blister rust, other diseases of North American forests, and general forest ecology to insure the application of biologically appropriate and sustainable management scenarios. Perhaps the most significant advances in understanding how host-pest interactions fit into the scope of biology have occurred in the last 10 years. This review focuses on an introduction to four recent developments that are fundamental to our understanding of how life originated, evolves, and functions. First, the almost universally accepted model of life, the Modern Synthesis (Huxley 1942), has provided biologists with a solid philosophical foundation for 70 years. In particular, this model has provided the theoretical basis for population genetics (Stern and Orgogozo 2009). Knowledge gleaned from complete genome (DNA) sequencing (see Mattick 2009) and the discovery of short, non-coding RNA transcripts (see Siomi and Siomi 2009) has eroded principal aspects of this venerable model and forced significant restructuring, which is currently in progress (Pigliucci 2010). Second, relevant new concepts (ecological immunity, plant immunity, and ecological speciation) born under the framework of the Modern Synthesis need updating. Integration of the genomic revelation into these concepts stands ready to revolutionize our understanding of forest ecology in general and plant disease in particular. Finally, this new lens will be focused on white pine blister rust in an attempt to uncover some new understanding.

Expanding the Modern Synthesis

Essentially all biological investigations of white pine blister rust were conducted from the philosophical perspective of the Modern Synthesis. A recent summary of the Modern Synthesis is quoted below to emphasize how a new model will contribute essential insight into forest biology.

> "1. Heredity occurs through the transmission of germ-line genes. Genes are discrete units that consist of DNA and are located on chromosomes.
>
> 2. Hereditary variation is equated with variation in DNA base sequence. Cases in which acquired variations appear to be inherited can all be explained in terms of variation in DNA.
>
> 3. Hereditary variation is the consequence of (i) the many random combinations of pre-existing alleles that are generated by the sexual processes; and (ii) new variations (mutations) that are the result of accidental changes in DNA. Hereditary variation is not affected by the developmental history of the individual. There is no "soft inheritance" (in which heritable variations are the result of environmental effects, use and disuse, and other factors).
>
> 4. Selection occurs among individuals that are, at almost all times, well-defined entities. The target of selection is almost always the individual, which may co-evolve with its symbionts and parasites. Although some role for group selection has been acknowledged, this form of selection is assumed to be of marginal significance in evolution. The community is rarely considered as a target of selection.

In: Fairweather, M. Comp. 2011. Proceedings of the 58th Annual Western International Forest Disease Work Conference; 2010 October 4-8; Valemount, BC. US Forest Service, AZ Zone Forest Health, Flagstaff, AZ.

[1]Emeritus Scientist, USDA Forest Service, Rocky Mountain Research Station, Forestry Sciences Laboratory, Moscow, ID gimcdonald@clearwire.net

5. Heritable variations have small effects, and evolution is typically gradual. Through the selection of individuals with phenotypes that make them slightly more adapted to their environment than other individuals are, some alleles become more numerous in the population. Mutation pressure is not an important factor in evolution. With a few exceptions, macroevolution is continuous with microevolution, and does not require any extra molecular mechanisms beyond those operating during microevolution.

6. Evolution occurs through modifications from a common ancestor, and is based on vertical descent. Horizontal gene transfer has minor significance – it does not alter the basic branching structure of phylogenetic divergence. The main pattern of evolutionary divergence is therefore tree-like, not web-like." (pp 389-390 in Jablonka and Lamb 2008)

Expansion of the Modern Synthesis Focuses on the Following Challenges to its Dogma.

"1. Heredity involves more than DNA. There are heritable variations that are independent of variations in DNA sequence, and they have a degree of autonomy from DNA variations. These non-DNA variations can form an additional substrate for evolutionary change and guide genetic evolution.

2. Soft inheritance, the inheritance of developmentally induced and regulated variations, exists and is important. Soft inheritance includes both non-DNA variations and developmentally induced variations in DNA sequence.

3. Since many organisms (including humans) contain symbionts and parasites that are transferred from one generation to the next, it may be necessary to consider such communities as targets of selection.

4. Saltational changes leading to evolution beyond the species level are common, and the mechanisms underlying them are begging to be understood. Macroevolution may be the result of specific, stress-induced mechanisms that lead to a re-patterning of the genome- to systemic mutations.

5. The Tree Of Life pattern of divergence, which was supposed to be universal, fails to explain all the sources of similarities and differences between taxa. Sharing whole genomes (through hybridization, symbiosis, and parasitism) and partial exchange of genomes (through various types of horizontal gene transfer) lead to web-like patterns of relations. These web-like patterns are particularly evident in some taxa (e.g. plants, bacteria) and for some periods of evolution (e.g. the initial stages following genome sharing or exchange)." (p 390 in Jablonka and Lamb 2008)

New challenges will continue to arise. In the meantime, these known challenges require additional discussion to examine why a reformulated Modern Synthesis carries important consequences for forest biologists.

Challenge Number 1– Genome and Epigenome

An accumulation of molecular and other data demonstrate that cells contain two sources of inheritance. The genome, home of DNA, and the epigenome, a non-DNA cellular memory that can pass through somatic cell lines, germ cell lines, and across generations. This heritable information is carried by two epigenetic signals (Bonasio et al. 2010). Cis epigenetic signals are physically associated with chromosomes (e.g. DNA and histone methylation marks) and trans signals are composed of various molecules partitioned by cytoplasm transfer. The cis signals are collectively known as the methylome and each cell type in an organism carries a unique signal that guides development and physiological function through gene regulation (see Rival et al. 2010). The methylome features sufficient conserved characteristics to permit construction of phylogenies (Zemach et al. 2010).

Quantitative and molecular genetics investigations of complex traits were based on the assumption of stable transmission of causative alleles encoded in the DNA genome (Johannes et al. 2008). This assumption was recently falsified in an experiment designed to control for DNA variation while maintaining variation in the epigenome. Heritability, arising from the epigenome, of 0.3 for height growth and 0.27 for flowering time was demonstrated in *Arabidopsis thaliana* (Johannes et al. 2009). The last few years have witnessed an explosion of papers investigating epigenomes, epigenomics, epialleles, and methylomes (see Baker 2010, Rival et al. 2010).

Challenge Number 2 - the "Ghost of Lamarck"

It is clear that short (18 to 200 nucleotides long) non-coding RNA transcripts, the genome, and the epigenome (see Ghildiyal and Zamore 2009; Lelandais-Briere et al. 2010), participate in sensing and "recording" information gleaned from abiotic and biotic environments to facilitate non-DNA inheritance of "acquired" traits (Hollick 2008; Bonduriansky and Day 2009). This phenomenon, also known as soft inheritance (Jablonka and Lamb 2008), is not well understood in plants. However, soft inheritance is currently known to influence regulation of all life-cycle phase transitions in plants, from seed germination to seed production, floral development, shoot apical meristem development, leaf development, vascular development, root development, abiotic and biotic (pests and competition) stress responses, and growth hormone signaling (Jung et al. 2009). In most of these cases, environmental induction is believed to be the source of variable expression (see Angers et al. 2010). In addition to the above participation in soft inheritance, small RNA transcripts travel throughout plants in the sap stream (Dunoyer et al. 2010; Zhang et al. 2009). Some specific examples of soft inheritance are: (1) juvenile growth rate in springtails (*Orchesella cincta*) (Ellers et al. 2008), (2) seed production (Whittle et al. 2009), and (3) flower production and height growth, as mentioned above, in *Arabidopsis thaliana*. Other examples are: (2) timing of bud phenology in Norway spruce (Yakovlev et al. 2010), (2) antibiotic resistance in bacteria is inherited via an epigenetic pathway (Adam et al. 2008), and (3) regulation of plant immunity responses to viral, bacterial, insect, and fungal pests of plants (Padmanabhan et al. 2009; Pandey and Somssich 2009). Finally, small RNA transcripts are upregulated by *Cronartium quercuum* f. sp *fusiforme* infection in *Pinus taeda* (Lu et al. 2007) but the epigenomic connection has not been investigated in this couplet. Symbiotic associations in plants also involve these regulatory pathways and epigenetic/RNA connections (Lelandais-Briere et al. 2010).

Challenge Number 3 – Transgenerational Transmission of Symbionts and Parasites

It may be necessary to consider trangesnerational communities as targets of selection. These situations may be of particular interest to the study of resistance as discussed below.

Challenge Number 4 – Rapid Evolution

Ecological speciation, also known as divergence-with-gene-flow, is currently a hot topic all its own (Via 2009). Studies illustrating detailed function of the epigenetic/RNA system in ecological speciation were not found. However, there is considerable support for the idea that environmentally driven genome-epigenome rearrangements could be responsible for divergence-out-gene-flow (Angers et al. 2010; Turner 2009; Aubin-Horth et al. 2009). Dramatic changes in the small non-coding RNA transcriptome are a known feature of artificial wheat hybrids (Lui et al. 2009) but details are lacking. An examination of the "long" transcriptome (transcripts longer than 100 nucleotides) of a species known to be expressing ecological speciation, the apple maggot, revealed a catalogue of potentially useful genes (Schwartz et al. 2009). Unfortunately these authors did not report on the "short" transcriptome (transcripts 18 to 30 nucleotides long). Study of the short transcriptome currently requires specific targeting through separation from the total transcriptome before sequencing (Zhang et al. 2010).

Challenge Number 5 - Web-of-Life or Tree-of-Life

Phylogenetic and phylogeographic theory will be significantly impacted if the Tree-of-Life morphs into the Web-of-Life, since these theories are built on the assumption of a last universal common ancestor (see Doolittle 2009; Koonin 2009).

Ecological Immunology

A field of study that weds ecology and immunology was initiated in the early 1990's from attempts to understand resistance to parasites and immune responses in birds and other vertebrates (Sadd and Schmid-Helmpel 2009). Recent realization that ecological immunology applies to all life has codified important concepts (Sadd and Schmid-Helmpel 2009) and led to the application of these concepts to all of biology (Schulenburg et al. 2009). Inclusion of plants was accelerated by the realization that plants also possess a fully functional immune system (Jones and Dangl 2006).

Plant Immunity

Upon attack, plants display an initial array of structural barriers and preformed antimicrobial metabolites. How these barriers work is not well understood, but it is clear that many pathogens can routinely penetrate these preformed defenses and activate systems of systemic immunity. The following short sketch of how plant immunity is currently understood to function is based on a recent review (Pieterse et al. 2009). Small

molecules known as phytohormones are essential signaling components that regulate plant growth, development, reproduction and survival. The primary defense phytohormones are salicylic acid (SA), jasmonates (JA), and ethylene (ET). However, abscisic acid (ABA), auxins, gibberellins, cytokinins and brassinosteroids may also play a role. The specific signal signature of the phytohormonal blend produced by a particular combination of host, trigger (i.e., pathogen, endophyte, symbiont, insect, parasitic plant, or wound), and environment activates a specific set of defense-related genes. Plant pathogens are classified as biotrophs (derive nutrients from living host tissue), necrotrophs (kill host tissue, then feed), and hemibiotroph (function both ways at different life cycle stages) and each triggers its own signal signature.

Basal or Quantitative Resistance

After initial penetration by a biotroph, necrotroph, or hemibiotroph, one of three types of recognition events occurs. First, pathogen-associated molecular pattern triggered immunity (PTI) initiates a cascade of basal defense gene activity that typically leads to (1) synthesis of callose and lignin to fortify the cell wall, (2) production of secondary metabolites such as phytoalexins, (3) accumulation of pathogenesis-related proteins, some of which can degrade fungal cell walls (Pieterse et al. 2009), and (4) stomata closure (Hou et al. 2009). Various pest infections, symbiotic associations, and wounding can prime basal resistance thereby increasing its effectiveness (Ahmad et al. 2010). Primed (Ahmad et al. 2010) and unprimed (Chen et al. 2010) basal defense appears to be the cellular mechanism leading to systemic induction of well known broad spectrum, durable, horizontal, or quantitative resistance mechanisms. Evidence from microarray experiments is beginning to show connections among basal resistance, priming and the epigenetic/small RNA gene regulatory network through regulation (positive and negative) of genes known to be associated with PTI in the soybean/*Phytophthora sojae* (Wang et al. 2010) and barley/*Puccinia hordei* (Chen et al. 2010) couplets. These studies also show that large numbers of genes (637 in soybean and 802 in barley) change state upon inoculation in resistant and susceptible comparisons. In general, plant hormones seem to be important in disease and defense against all microbial attacks (Bari and Jones 2009). In the barley/rust couplet, infection induced significant changes in a transcription factor (HvERF4) known to be active in defense pathways relating to ethylene, jasmonic acid, and abscisic acid (Chen et al. 2010). Finally, degree of basal resistance expression is influenced by temperature through the regulation of a defense-related proteins and/or impacts on SA signaling (Zhu et al. 2010). Expression of quantitative disease resistance is often associated with various aspects of growth and development such as flowering time, stomata development, and ability to repel water (Poland et al. 2008).

Major Gene or Qualitative Resistance

If a specific host-pathogen couplet has a coevolutionary history, the pathogen may acquire effector molecules that promote virulence to initiate effector-triggered susceptibility (ETS). At some point in their coevolutionary history, the host acquires resistance proteins that sense the pathogen effector. This action initiates a secondary response termed effector-triggered immunity (ETI). ETI produces a burst of reactive oxygen species that initiate the well-known programmed hypersensitive cell death, a well known indicator of major gene resistance.

Endophytes and Symbionts

Beneficial microbes also communicate with the host, but trigger a phenotypically similar yet distinct kind of systemic immunity called induced systemic resistance (ISR). With ISR, signal molecules are JA and ET; the induced condition primes for defense, not outright activation of defense. Pathogens, insects, and wounding can trigger a JA- and ET-mediated response. However, crosstalk among the phytohormones is common and many details are lacking. The above model of host-pest interaction has been developed through the use of Arabidopsis and a few other model systems. Many aspects of the system have been observed in various species of conifers and angiosperm trees (Eyles et al. 2010).

Common Machinery

The epigenetic/small RNA gene regulatory network appears to modulate ETI as well PTI (Padmanabhan et al. 2009). The major players (microRNA and small interfering RNA) also participate in epigenomic interactions leading to soft inheritance. Multiple specific miRNA transcripts all functioning in different manners can be produced by a single micro RNA gene (Zhang et al. 2010), and there may be thousands of these micro RNA genes in the “junk” DNA. Biogenesis of small interfering RNA (siRNA) transcripts is even more complex. In this case, sense

and antisense transcripts are derived from overlapping regions between adjacent genes, pseudogenes, and distant compatible genes (Ghildiyal and Zmore 2009; Muro and Andrade-Navarro 2010; Rival et al. 2010). Once PTI or ETI is activated at the site of infection, SA often spreads systemically to produce systemic acquired resistance (SAR). This long-lasting and broad-spectrum disease resistance is generally triggered by biotrophs. SAR initiated by necrotrophs and wounding is most often triggered by JA or ET.

Tolerance

Tolerance (see Raberg et al. 2009) is part of the ecological immunity paradigm with its own physiology and genetics. This concept, which applies to plants and animals, is difficult to understand because quantification requires assessment of reaction norms for host fitness across a range of pest burdens. Degree of tolerance is indicated by differences in slopes of the obtained reaction norms and equal reaction norm slopes are a measure of host general vigor. No examples of functioning epigenetic/small RNA gene regulation networks were found for tolerance reactions to include in this review.

Ecological Speciation

Conventional wisdom states that gene flow will homogenize adjacent populations so strongly that divergence in the absence of geographic separation will not occur. But, the sheer number of examples of phenotypic divergence between adjacent populations (suture zones) raised the need for an explanation. The following sketch is based on a recent review of "divergence-with-gene-flow" (Via 2009). Current understanding of speciation genetics is almost entirely based on long-range retrospective studies conducted from the viewpoint of "good species". This so-called spyglass model has culminated in the dominance of the idea that reproductive isolation (no gene flow) is essential for speciation to happen and that geographic isolation is a necessary condition to stop gene flow. A new approach, called the magnifying glass model, is based on the study of how genetic x environment interactions can lead to incipient reproductive isolation among ecotypes or races in the absence of geographic isolation. The obvious result of having two evolutionary avenues is that ecological speciation can happen rapidly with the development of "ecological barriers" or classic speciation can happen slowly with geographic isolation. Divergence-with-gene-flow can occur only if reproductive barriers developed within the genome are strong enough to maintain phenotypic differentiation. This condition means strong selection and rapid divergence can happen when selection is directed at multiple traits bearing on resource or habitat use. In this case, selection is also strong against migrants and hybrids. Studied examples indicate that conditions suitable for development of divergence-with-gene-flow are common in host-pest interactions but are not limited to these interactions. Additional important aspects not recognized by Via (2008) are the roles gene expression and epigenetics can play in ecological speciation. However, others (Wolf et al. 2010) suggested that integration of gene network thinking into speciation genetics may have an impact similar to that of Mendelian genetics on Darwin's original framework.

Ecological Immunology x Ecological Speciation

Finally, a brief examination will show how critically important the environmental context of host-pest interactions is for determining specific outcomes. This brief synopsis is largely based on a recent review of immunity in a variable world (Lazzaro and Little 2009). Given that activation of immunity incurs physiological costs, the whole organism is involved when a part is attacked. Further, the strength and duration of the immune response is heavily influenced by the overall condition of the potential host. Important variables are abiotic environment, genotype x environment interactions of host and pest, host genotype x pathogen or symbiont genotype interaction (biotic environment), and pleiotropic constraints. The potential importance of these interactions is illustrated by the results from a study reported in Lazzaro and Little (2009). A three way host genotype x pest genotype x environment (presence or absence of rhizobacteria) interaction showed that as much as 42 percent of the barley performance and 32 percent of the aphid performance was explained by the 3-way interaction.

To understand why outcomes of such associations are so difficult to predict, consider a hypothetical host x symbiont x pathogen expression of basal resistance (all eukaryotes) in two environments given that each of the living participants were cloned to ensure the same interacting sets of DNA in both environments. To examine how these interactions might function, we need a little more background. We know from our discussion above that about 600 host genes respond to infection. Let us assume 200 additional host genes respond to the symbiont, that 100 pathogen and 100 symbiont genes also respond. Thus, 1000 genes are

interacting. Not all these responders encode proteins. So assume that 0.8 (800 genes) represent non-coding elements associated with both genome and epigenome which are differentially expressed by cell type (Baker 2010). There are eight types of non-coding elements (Alexander et al. 2010), of which transcription factors, small interfering RNA, micro RNA, and pseudogenes are the most well known. In addition, expression varies by cell type and number of cell types (ca. 4 for fungi and 20 for conifers) varies by complexity of the organism (Alexander et al. 2010). Alternative splicing of messengerRNA from coding genes leads to multiple expressions of single genes in eukaryotes (Nilsen and Graveley 2010); therefore 200 (coding genes) is multiplied to about 600 expressed states (assume 3 alternative forms per gene). Next, consider that each organism has its own genotype x environment, genotype x development, and genotype x ontogeny interaction (Kliebenstein 2010). Also individual microRNA genes can generate multiple functional transcripts (Zang et al. 2010), and pseudogenes can participate in the production of natural antisense transcript gene regulators (Muro and Andrade-Navarro 2010). Even with this simplified level of complexity, it is evident that we are a long way from understanding basal resistance in conifers!

Potential Blister Rust Answers and Pitfalls

Perspectives provided by an expanded Modern Synthesis (i.e. evolution, development, and function rise from gene regulatory networks that are formulated via the interaction of genomes, epigenomes, and environment) can further inform our understanding of white pine blister rust (WPBR).

Why do Plantations Exhibit so Many Pest Problems?

As discussed above, the realization is growing that gene regulatory networks sense information from the environment and store gene regulation profiles for the current as well as future generations. We expect profile construction to start at embryogenesis and continue to develop until at least reproductive maturity. In long-lived organisms, the gene regulation profile may change year by year to ensure an adequate response to accumulated lifelong stressors – abiotic as well as biotic. Over a few generations, these gene regulation profiles may become incorporated into the genome so as to facilitate rapid "adaptation" to environmental change (e.g., ecological speciation). Populations of long-lived tree species may be especially fine-tuned to specific populations of endophytes, symbionts, pests, and competitors as well as multiple factors in the abiotic environment (see Bossdorf et al. 2008). We have already examined how multiple levels of genetic and environmental interaction can produce an incredible range of outcomes. Artificial reforestation methods could cause much greater problems than currently realized because many sources of interaction are ignored under current practice. Disruption of local gene regulation profiles in natural hybrids could be the driving force in ecological speciation (Wolf et al. 2010) and artificial (breeding) and natural (offsite) hybrids likely contribute to plantation problems in the same fashion. Since forest trees have extended development periods, it is also possible that many aspects of nursery (e.g., growing seedlings in mismatched biotic and abiotic environments) and planting practice might impart negative influences for the life of a plantation. Thus, one would expect intermittent expression of significant pest and/or environmental problems at a local scale for off-site (i.e., planted) plantations. The application of the expanded Modern Synthesis paradigm also leads to two additional conclusions: (1) global climate change would likely cause more disruption in plantations than in natural stands, and (2) development and interpretation of experimental plantations requires much caution. Next up is a case in point.

Why did Blister Rust Resistance Fail at the Merry Creek and Hold Firm at Gletty Creek?

Large bulk-seed lots of northern Rocky Mountain western white pine (WWP) representing: (1) open-pollinated controls, (2) full-sib, 1st generation crosses (phenotypically resistant parents), 2nd generation full-sibs (1st generation crosses), and full-sib back crosses (1st generation x original parents) were divided into two groups and planted at two sites (Merry Creek 1970, Gletty Creek 1972) (Bingham et al. 1973). After 26 years of exposure, all sources planted at Gletty Creek were still below expected infection threshold; whereas, all sources planted at Merry Creek dramatically exceeded expected infection thresholds (McDonald and Decker –Robertson 1998; McDonald et al. 2004). Was this failure attributable to a resistance gene collapse or some other cause? A major gene for resistance to WPBR is known to be present in some WWP subpopulations (Kinloch et al. 2003), but neither the host resistance gene nor the pathogen virulent gene are known to exist within the range of the WWP subpopulation used in these plantations (Kinloch et al. 2003; Kinloch et al. 2004).

Several lines of evidence indicate that the population tested at Merry Creek and Gletty Creek is expressing quantitative (basal) resistance (McDonald and Decker –Robertson 1998; McDonald et al. 2004).

Basal Resistance in WWP:

Investigation of quantitative resistance expression in WPBR has deposited several clues about its nature. The updated Modern Synthesis implies that understanding the failure at Merry Creek requires consideration of host developmental stages, cell types, influence of the physical environment, and associated symbionts. Since stomata are the infection court and white pines produce 3 kinds of needles or organs (i.e., cotyledons, primary needles, and secondary needles), which each supports at least 4 cell types, as many as 12 methylomes could be involved, in addition to host and rust genetics. Cotyledons (i.e., embryonic leafs) have been used to assess qualitative resistance to WPBR (Kinloch et al. 2004), but not basal resistance.

We will compare results from inoculation tests at Moscow, ID in 1964, 1966 and 1967, 1968, and 1970. Primary needles (i.e., first-year growth) were inoculated and inspected in several tests due to the tendency for delayed germination. Most often, seedlings were inoculated after bud set in their 2nd year when only secondary needles were present. Some combinations of years, sites, and/or families led to a second flush of leader growth that also supported primary needles; however, these are customarily removed before inoculation. Resistance observed in the Merry Creek families and other sources of WWP (McDonald and Hoff 1970b, 1971; Hoff and McDonald 1971; McDonald et al. 2004; Hoff et al. 1980) are likely basal. The six resistance phenotypes defined in these studies, listed in order of occurrence along the plant-development/cell-type pathway, are: (1) needle-spots-only in primary needles (NOSP), (2) rust-free secondary needles (RF), (3) reduced-needle-lesion-frequency in secondary needles (RNLF), (4) needle-spots-only in secondary needles (NSOS) composed of premature-needle-shed of secondary needles (PNS) and fungicidal-short-shoot (FSS), and (5) partial-girdle resistance (PG) expressed in stems and branches.

Needle-Spots-Only Primary (NSOP) vs. Secondary Needles (NSOS)

Influence of foliar type at infection on canker presence 3 years after inoculation was assessed in the 1966 test (data on file Moscow FSL) and the 1970 test (Hoff et al. 1980). In the 1970 test (6 years in greenhouse and lath house), 23 percent of 35 F1 seedlings supporting needle spots on primary foliage were clean (no rust) after 3 years and 36 percent of 1,108 seedlings with needle spots on secondary needles (mixture of 2- and 3-year-old plants) were clean (Hoff et al. 1980). In the 1966 inoculation (outside Moscow ID), susceptible (open-pollinated infected parents) and resistant (F_1 and F_2) seedlings were compared (Moscow FSL data on file). About 60 seedlings in each primary and secondary class for each seed lot (i.e., F_2-primary-full sib family) supported needle infections at 12 months. Percent clean in each class was determined 3 years after inoculation. Results, in percent clean for the three stocks (about 250 seedlings/cell), were control (7 percent), F_1 (10 percent), and F_2 (18 percent) for first year seedlings, and 22, 34, and 65 percent, respectively, for second year seedlings. Individual F_1 and F_2 families, 60 individuals per cell, exhibited dramatic differences in the primary vs. secondary comparison. Family 242 x 224 (F1) showed no difference (15 percent vs. 13 percent); 129 x 224 showed a large difference (0 percent vs. 38 percent); and 208 x 241 showed another large difference (8 percent vs. 39 percent). Family 58x25-9 X 18x17-9 (F2) changed from 7 percent to 83 percent, while 58 x 25-9 X 22x1-4 changed from 24 percent to 28 percent. Relatively small differences between the tests for F_1 stock (NSOP 23 percent vs. 10 percent and NSOS 36 percent vs. 34 percent) may be noteworthy because of the dramatic difference in growth regimes. The 1970 test was conducted under controlled conditions for the entire 6-year duration, in which the first 3 years alternated between greenhouse and lath house and the last 3 years were entirely in the lath house. In the 1966 test, seedlings were outside for the entire duration of the test. Does this indicate some development x environment interaction for NSOP and none for NSOS? On the other hand, the individual family results indicate a highly significant genetic (genome and epigenome?) x development interaction.

Reduced Needle Lesion Frequency (RNLF) Resistance

This mechanism was reported for 80 full-sib F_1 families inoculated in their 2nd growing season in 1966 (Hoff and McDonald 1971) and 120 different F_1 families inoculated in 1970 (McDonald et al. 1991). Both tests were conducted on seedlings grown outside at Moscow, ID. The average spots/meter of needle length in the 4 lowest and 4 highest families for

the 1966 inoculation (spores cast not reported) was 1.75 and 14.25, respectively, or an 8.1x difference and a mean of 7.5. The same data for the 1970 inoculation (2,500 spores/cm^2) was 2.15 and 18.4 or 8.6x differences and a mean of 8.4. The species difference reported for secondary needles in the 1970 greenhouse growth and inoculation test (4,900 spores/cm^2) was 0.1 (*P. peuce*) and 28.0 (*P. ayachuite*) or a 280x difference. The mean spotting frequency of WWP (F_1) in the greenhouse test was 5 spots/meter of needle. In another experiment conducted in the greenhouse/lath house at Moscow, 11 two–year-old WWP and *P. lambertiana* families obtained from the Dorena Program located in Oregon were quantitatively inoculated in large settling towers featuring rotating basidiospore delivery beds (McDonald et al. 1991). Inoculation efficiency was calculated on the basis of stomatal area exposed to spore cast as determined by multiple spore traps in a small area. Spores were delivered, by design, at 8 levels varying from 600 to 18,000 spores/cm^2. Yet, infection efficiency was relatively stable and the families varied from 0.1 to 1 relative to the highest spotting family, again a 10x difference. Others demonstrated that stomatal area and contact angle of water drops formed on secondary needles differed significantly in comparison of susceptible and resistant materials (Woo et al. 2001). Another study reported that stomata/row, stomatal shape, stomatal density, mean stomatal area, wax degradation, water-contact angle with wax and contact angle without wax all vary significantly in the same F_2 seed lot grown at three different nurseries (Woo et al. 2002). The surface water vs. basal resistance connection has been made by others (Poland et al. 2009). In summary, RNLF would seem to be a relatively stable trait that could influence amount of rust infection in various WWP families and perhaps in differing white pine species. However, the needle-trait studies and the large differences in level of rust-free seedlings in F_1 WWP grown and inoculated under varying conditions raise significant cautionary flags.

Needle Spots Only (NSO) and Partial Girdle (PG) Resistance

Tests inoculated in 1964, 1968, and 1970 also investigated a resistance mechanism termed needle-spots-only, hypothesized to be a composite of two mechanisms called (1) premature-needle-shed (PNS), where infected needles were shed before the rust penetrated the short shoot (McDonald and Hoff 1971), and (2) fungicidal-short-shoot (FSS) where infection failed to penetrate the short shoot (Hoff and McDonald 1971). In the 1964 test, 99.5 percent of the seedlings exhibited needle infections and only the composite mechanism was delineated on F_1 and susceptible stocks 2 years post infection. Of 2,878 F1 seedlings, 19 percent expressed resistance, and of 345 open-pollinated control seedlings, 10 percent were classified resistant. Results for the 1968 inoculation were based on 546 open-pollinated controls, 2,876 F1s, and 3,061 F_2s. Two new categories were added, rust-free (RF), seedlings without symptoms, and a bark-reaction labeled partial girdle (PG) wherein the cankers appeared to be cleared from stems and/or branches. Rust-Free seedlings were removed from the totals to calculate the remaining percentages in both tests. PNS equaled 15 percent (control), 20 (F_1), and 48 percent (F_2); FSS equaled 2 percent (control), 5 percent (F_1), and 12 percent (F_2); and PG equaled 2 percent (control), 8 percent (F_1), and 7 percent (F_2) (Hoff et al. 1973). The 1970 test (Hoff et al. 1980) included 18 species of white pine, but only F_1 WWP stock is included in this discussion. Results were 21 percent (PNS), 4 percent (FSS), and 11 percent (PG). In regard to PNS, FSS, and PG in the F_1 families, the three tests compare very favorably. We conclude that NSO was stable under the range of abiotic (and maybe biotic) conditions experienced under experimental conditions up to about 6 years of age, and that infection in F_1 stock should plateau at 65 percent (e.g. 1-PNS (20 percent) + FSS (5 percent) + PG (10 percent) = 65 percent). From these results, open-pollinated controls should plateau at about 80 percent and F_2 at 35 percent. Predictions (1968 test) were control = 81 percent, F_1 = 67 percent, and F_2 = 34 percent infection (Hoff et al. 1973).

Rust-Free Resistance (RF)

Occasionally rust-free seedlings, which showed up in the large-scale inoculation tests, were treated as escapes at Moscow. On the other hand, given the possibility that induced stomatal closure can enhance basal resistance (Hou et al. 2009), the issue should be revisited. No information was found in the materials supporting this review pertaining to RF primary needles. In the 1968 test, RF equaled 4 percent (control), 1 percent (F_1), and 13 percent (F_2). In the 1970 test, RF equaled (24 percent) for F_1 stocks. Thus, RF in F_1 families increased dramatically (i.e., 1 percent to 24 percent), when the inoculation was conducted within the bounds of a greenhouse and lath house, while expression of other mechanisms changed little (see above).

NSO in the Real World

Stocks developed from the northern Rocky Mountain WWP breeding program were placed in test plantations in addition to Merry Creek and Gletty Creek. Early stock representing F_1 full-sib families from phenotypically resistant parents were planted with open-pollinated controls at Priest River and Deception Creek Experimental Forests in north Idaho (McDonald et al. 2004). After about 45 years of repeated assessments, disease progress curves showed that infection in F_1 families reached a plateau of 40 percent at 25 years and remained stable for another 17 years at both sites. This performance was better than expected. The controls planted at the Experimental Forests also presented surprises. At 45 years, disease progress curves for both were still trending upward, but the expected infection plateaus (estimated by curve fitting) hint at a rapid increase in resistance phenotypes in the susceptible population. Open-pollinated seed was collected from the same cankered trees in 1953 and 1955 and used as control stock for the 1957 and 1959 plantings. Estimates of infection plateaus generated by curve fitting were 72 percent and 83 percent at Priest River and 77 percent and 89 percent at Deception Creek for the two collection years respectively. Both sites indicate a 10 percent gain in resistance from susceptible parents in two years and both plantings fit with nursery test expectations. Was this gain caused by changes in gene frequency (Modern Synthesis) or soft inheritance? After 26 years, at Gletty Creek (sister planting of Merry Creek), control, F_1, and F_2 stocks, supported 94, 46, and 20 percent infection, respectively (Moscow FSL data on file). Since these trends appear to be following those at Priest River and Deception Creek, I conclude that Gletty Creek is meeting or exceeding expectation of resistance.

The northern Rockies stocks were also planted in California and British Columbia (BC). At the Happy Camp site in northern California, control, F_1, and F_2 families were planted in the early 1970s and inspected periodically (Kinloch et al. 2008). Since none of these WWP materials express the major gene, this aspect of WPBR at Happy Camp will be ignored in this discussion. First, we must address the matter of expected resistance. As discussed above, individual control lots, F_1 full-sib families, and F_2 full-sib families can vary widely in expected levels of resistance as judged from inoculation tests of seedlings supporting only secondary needles. Ranges of variation in expected percent infected (i.e., percent infected = 1-tested percent clean) observed in a limited data set from the 1966 test are: control (14 to 34 percent), F_1 (5 to 49 percent), and F_2 (58 to 83 percent) (Moscow FSL data on file). Since the Happy Camp data are presented as simple scatter plots with uneven time intervals, comparison to disease progress curves is complex. Thus, I will focus on the apparent infection asymptote demonstrated in the published scatter plots (Kinloch et al. 2008 p72). Control-lot asymptotes of 100 percent and 90 percent meet nursery expectation. Three F_1 families show 70, 60, and 90 percent asymptotes and the expected range is 51 to 95 percent. Thus, the F_1 families meet the nursery test expectation. Two F_2 families reached asymptotes of 35 percent and 40 percent, which is within the expected range of 17 to 42 percent.

Idaho F_2 stock and local controls were planted at 2 coastal British Columbia sites, one low elevation and one high elevation (Hunt and Meagher 1989). After 12 to 13 years exposure to WPBR, infection levels were, low-elevation control 52 percent, low-elevation F_2 75 percent, high-elevation control 21 percent, and high-elevation F_2 10 percent. After 20 years of exposure at an interior BC site, F_2 stock was 35 percent infected while controls were 100 percent infected (Hunt 2005). Test plantings of F_1 and F_2 stock in California (1 site), Idaho (3 sites), interior BC (1 site), and high elevation coastal BC (1 site) have performed to expectation or better. Two sites, low-elevation coastal BC and north Idaho, failed to meet expectation. Some production plantations of F2 stock have also exhibited higher than expected levels of infection (Schwandt and Ferguson 2003). In light of the expanded Modern Synthesis we can hypothesize these failures are triggered by the environment. Also, Hunt (2004) discusses the effects of environment on expression of blister rust resistance and, although numbers are small, indicates that environmental factors may be implicated of the failure of F_2 NSO resistance. Hunt (2005) compared WWP seedlings and grafts and low and high elevation coastal sites and concluded that genetic x environment interactions influenced expression of resistance.

Direct influence of environment on RNLF expression comes from a couple of “accidental experiments” reported by Woo et al. (2004). Two seed lots grown at two different nurseries inoculated in a common inoculation facility and then returned for 3 years of development to their original nurseries. One lot was expected to express a relatively high level of

resistance and the other a low level. Mortality in the resistant stock was 48 percent 3 years post infection, while the susceptible stock exhibited 30 percent mortality. This prompted an experiment designed to control for genetics and environment. Bulk F_2 seedlings were grown at two different nurseries prior to infection and were scheduled to be inoculated in September of 1999 at a common facility. Seedlings from one nursery were placed in cold storage in December of 1998. However, due to an oversight, the seedlings were not removed from cold storage until early August of 1999. Meanwhile, the other lot was subjected to a normal cycle. At 5 months post infection, seedlings exposed to extended cold storage, (immature secondary needles) exhibited 100x the infection efficiency of the mature needles – a level indicating low RNLF in F_2 stocks. The overall conclusion is that the failure of resistance at Merry Creek was probably due to an environmentally triggered collapse of basal resistance. It seems WPBR basal resistance is a classic example of a "plastic immunity response" as described for the concept of ecological immunity (Sadd and Schmid-Hempel 2008).

White Pine Blister Rust Phytohormone Interaction

Materials from the 1966 progeny test were also used to investigate the interaction of WPBR infection, rooting medium, and hormone application on rooting of WWP (McDonald and Hoff 1970a). Single-needle fascicles were harvested from each of 12 individual outdoor-grown seedlings from each of 104 WPBR-resistant families in early March at 5 months post inoculation. Needle infections were not visible at harvest; however, 99.5 percent of the seedlings exhibited needle spots by early June, indicating that the uninfected needles were most likely derived from infected seedlings. WPBR infection depressed rooting by 40 percent in half-sib family 17. Tester 17 was later shown to segregate in selfed families (McDonald and Hoff 1971) as a trait controlled by two recessive genes. Meanwhile, the remaining half-sib families (19, 22, and 58) exhibited depressed rooting by 11, 9, and 19 percent, respectively. When selfed, these families segregated in a single recessive pattern. From the perspective of an expanded Modern Synthesis, these results probably indicate a connection between WPBR infection and hormone metabolism. On the other hand, this genetic hypothesis has been questioned (Kinloch et al. 2008; Hunt 2004). In fact, given current understanding of transcriptome behavior associated with basal resistance, I also question this genetic hypothesis. Further, it must be said that disproving a genetic hypothesis about a trait does not negate the reality of the phenotype. So, what does depression of rooting by WPBR needle lesions tell us about how ecological immunology functions in WPBR? Since the rooting depression seems not to be expressed in healthy needles obtained from plants supporting a needle infection, systemic signaling may not occur until penetration of cell types located in the stem. But, how would this observation fit with the possibility of needle endophytes priming basal resistance as discussed above. Significant interactions among the WPBR fungus and naturally occurring endophytes appears to be an important aspect of blister rust biology (Ganley et al. 2008). Many root zone and stem pathogens and symbionts could also be present and functioning with the aid of a systemic signaling system.

Some Additional Questions

The demonstrated existence and importance of the epigenome allows us to re-examine persistent questions that are associated with WPBR studies. These questions include the following: Is the common occurrence of overdispersion (maximum infection incidence often fails to reach unity) in WPBR epidemics (McDonald et al. 2005) related to the abiotic environment, induced immunity, endophytes, soil conditions, or some combination of these factors? Why did a high-severity burn during site-preparation at Merry Creek result in equal infection rate increases in control, F_1, and F_2 test populations relative to a lower severity burn (McDonald and Decker-Robertson 1998)? Can burn treatments influence host resistance in a manner similar to biochar, which has been shown to induce resistance to both biotrophic and necrotrophic pathogens on both tomato and peppers (Elad et al. 2010)? In a general sense, is an appropriate local natural control population required to insure reliable results from any forest management experiment? Given our current understanding of plant immunity, does the existence of major-gene resistance in the pine host imply a significant coevolutionary history between white pines and *Cronartium ribicola*? Would a reexamination of historic data from a new perspective reveal new awareness about WPBR interactions? Regarding ongoing screening and breeding programs of white pines, how confident can we be that subpopulation structures of pine have not "evolved" rapidly enough by way of ecological speciation to ultimately cause significant host genotype x pathogen genotype x environment interactions? Does awareness of many new alternate

hosts (Mulvey and Hansen 2011, Kattera and Hiltunen 2010) indicate pine genotype x Ribes genotype x Pedicularis genotype x Castilleja genotype x rust genotype interactions should be expected? Do such complex host-pathogen-environment interactions reflect a mature pathosystem? Do implications arising from soft inheritance indicate that more emphasis should be placed on natural regeneration of conifer forests? Are there serious negative consequences to developing resistant host populations through creation of artificial hybrids screened for resistance under artificial conditions that do not reflect the conditions where the resulting populations are deployed? Results from an initial range-wide study of AFLP molecular markers in WWP (Kim et al. 2011) and analysis of host growth and blister rust expression in a common garden experiment (McDonald ms in progress) together illustrate a potentially complex and interactive WWP population substructure.

Summary - A Management Dilemma

The expanded Modern Synthesis poses management dilemmas, such as (1) selection and deployment of natural quantitative or forced qualitative resistance, (2) deployment of potentially disruptive stock from a geographically broad breeding program vs. prudent local management of natural populations, and (3) utilization of large economically efficient screening facilities vs. sophisticated screening designed to yield stock that fits subpopulation boundaries. Basal resistance, while sometimes subject to environmentally triggered breakdown, might still be the best choice as indicated by its major role in dampening pest activity in natural forests. This observation fits with “optimal immune defense”, an important concept of ecological immunity. Qualitative resistance, notoriously susceptible to virulence variation, may be a poor option for forest trees judging from its relatively minor role in forest systems and, according to the “usage costs of defense” principle of ecological immunity, this option would likely incur higher fitness costs than basal resistance. Increasing knowledge about ecological speciation and the complexities of genome x epigenome x environment interactions indicate local management of local populations may result in more stable forest ecosystems. Further, the biological paradigm encompassed in a new Synthesis argues that large screening facilities will most likely produce maladapted populations due to the sheer complexity of controlling all the important sources of variation. Approaches discussed will also apply to understanding and managing the heightened pest problems expected with climate change (Grulke 2011).

Will the new Synthesis apply to our target populations? A good foundation for molecular investigations has been developed and was recently reviewed (Richardson et al. 2010) from the perspective of classic Modern Synthesis. Some important factors associated with the new paradigm that have been observed are: microRNAs in lodgepole pine (Morin et al. 2008); small RNAs in Norway spruce, white spruce, eastern white pine, and Douglas- fir (Dolgosheina et al. 2008); and microRNAs and epigenetic inheritance were associated with climate adaptation of Norway spruce (Yakovlev et al. (2010). As already mentioned, microRNAs were shown to be regulated by fusiform rust infection in loblolly pine (Lu et al. 2007). The CC-NBS-LRR subfamily of proteins, known to be associated with plant immunity, was shown to be active in WWP with possible links to qualitative resistance (Liu and Ekramoddoullah 2007). Eighty-three members of the WRKY family of transcription factors, associated with plant immunity, were found in WWP and one member was linked to a major resistance gene (Liu and Ekramoddoullah 2009). Another examination of signaling and pathogenesis-related proteins in WWP revealed that levels of a pathogen-related protein were increased by WPBR infection, wounding, and methyl jasmonate application (Ekramoddoullah et al. 2006).

Limitations imposed by the gene-centric Modern Synthesis have significantly constrained depth of analysis of WPBR evolution and ecology in particular and forest biology in general. A major weakness is lack of awareness regarding the impact of interaction between genotype, development, and soft inheritance. A theory incorporating epigenomes, gene regulatory networks, soft inheritance, ecological immunity, and ecological speciation should be embraced. As suggested above, an expanded Modern Synthesis can reveal management implications of large effect. I propose using the new paradigm to: (1) conduct a thorough review of current breeding and forest management programs (e.g., Schoettle and Sniezko 2007; King et al. 2010); (2) review existing literature using a brighter light; (3) re-evaluate remaining data archives to achieve improved insight; (4) develop new study designs; and then (5) initiate pilot studies in conifers, while awaiting sequencing of a conifer

genome, directed at transcriptome discovery and function using 3rd generation single-strand sequencing (see Morozova et al. 2009; Mamanova et al. 2010). In conclusion, new theory, a 100-year legacy of experimentation and observation, powerful new tools, and a substantial need for new knowledge all argue for rapid advance toward a more comprehensive understanding of WPBR coevolution. The goal, of course, is sustainable management of conifer ecosystems worldwide.

REFERENCES

Adam, M., Murali, B., Glenn, N., Potter, S. 2008. Epigenetic inheritance based evolution of antibiotic resistance in bacteria. BMC Evolutionary Biology 8:52-63.

Ahmad, S., Gordon-Weeks, R.J., Pickett, J., Ton, J. 2010. Natural variation in priming of basal resistance: from evolutionary origin to agricultural exploitation. Mol. Plant Path. 11:817-827.

Angers, B., Castonguay, E., Massicotte, R. 2010. Environmentally induced phenotypes and DNA methylation: how to deal with unpredictable conditions until the next generation and after. Mol. Ecol. 19:1283-1295.

Aubin-Horth, N., Renn, S.C. 2009. Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. Mol. Ecol. 18:3763-3780.

Alexander, R.P., Fang, G., Rozowsky, J., Snyder, M., Gerstein, M.B. 2010. Annotating non-coding regions of the genome. Nat. Rev. Genetics 11:559-571.

Baker, M. 2010. Epigenome: mapping in motion. Nature Methods 7:181-185.

Bari, R., Jones, J.D.G. 2009. Role of plant hormones in plant defense responses. Plant Mol. Biol. 69:473-488.

Bingham, R.T., Hoff, R.J., McDonald, G.I. 1973. Breeding blister rust resistant western white pine. VI. First results from field testing of resistant planting stock. USDA Forest Service Research Note INT-179, Intermountain Forest and Range Experiment Station, Ogden, Utah. 12p.

Bonasio, R., Tu, S., Reinberg, D. 2010. Molecular signals of epigenetic states. Science 330:612-616.

Bonduriansky, R., Day, T. 2009. Nongenetic inheritance and its evolutionary implications. Ann. Rev. Ecol. Evol. Syst. 40-103-125.

Bossdorf, O., Richards, C.L., Pigliucci, M. 2008. Epigenetics for ecologists. Ecology Letters 11:106-115.

Chen, X., Niks, R.E., Hedley, P.E., Morris, J., Druka, A., Marcel, T.C., Vels, A., Waugh, R. 2010. Differential gene expression in nearly isogenic lines with QTL for partial resistance to *Puccinia hordei* in barley. BMC Genomics 11:629.

Dolgosheina, E.V., Morin, R.D., Aksay, G., Sahinalp, S.C., Magroni, V., Mardis, E.R., Mattsson, J., Unrau, P. 2008. Conifers have a unique small RNA silencing signature. RNA 14:1508-1515.

Doolittle, W.F. 2009. The practice of classification and the theory of evolution, and what the demise of Charles Darwin's tree of life hypothesis means for both of them. Phil. Trans. R. Soc. B 364:2221-2228.

Dunoyer, P., Schott, G., Himber, C., Meyer, D., Takeda, A., Carrington, J., Voinnet, O. 2010. Small RNA duplexes function as mobile silencing signals between plant cells. Science 328:912-916.

Ekramoddoullah, E.K.M., Liu, J.-J., Zamani, A. 2006. Cloning and characterization of a putative antifungal peptide gene (Pm-AMP1) in *Pinus monticola*. Phytopathology 96:164-170.

Elad, Y., David, D.R., Harel, M.H., Borenshtein, M., Kalifa, H.B., Silber, A., Garber, E.R. 2010. Induction of systemic resistance in plants by biochar, a soil-applied carbon sequestering agent. Phytopathology 100(9):913-21.

Ellers, J., Marien, J., Driessen, G., Van Straalen, N. 2008. Temperature-induced gene expression associated with different thermal reaction norms for growth rate. J. Exp. Zool. (Mol. Dev. Evol.) 310B:137-147.

Eyles, A., P. Bonello, R. Ganley and C. Mohammed. 2010. Induced resistance to pests and pathogens in trees. New Phytologist 185: 893-908.

Ganley, R.J., Sniezko, R.A., Newcombe, G. 2008. Endophyte-mediated resistance against white pine blister rust in *Pinus monticola*. Forest Ecology and Management 255:2751-2760.

Ghildiyal, M., Zamore, P. 2009. Small silencing RNAs: an expanding universe. Nat. Rev. Genet. 10:94-108.

Grulke, N.E. 2011. The nexus of host and pathogen phenology: understanding the disease triangle with climate change. New Phytologist 198:8-11.

Hoff, R.J., McDonald, G.I. 1971. Resistance to *Cronartium ribicola* in *Pinus monticola*: short shot fungicidal reaction. Can. J. Bot. 49:1235-1239.

Hoff, R.J., McDonald, G.I., Bingham, R.T. 1973. Resistance to *Cronartium ribicola* in *Pinus monticola*: Structure and gain of resistance in the second generation. USDA For. Serv. Res. Note INT-178, 7p.

Hoff, R.J., Bingham, R.T., McDonald, G.I. 1980. Relative blister rust resistance of white pines. European J. Forest Pathology 10: 307-316.

Hollick, J. 2008. Sensing the epigenome. Trends in Plant Science. 13:398-404.

Hou, S., Yang, Y., Zhou, J-M. 2009. The multilevel and dynamic interplay between plant and pathogen. Plant Signaling and Behavior. 4:283-293.

Hunt, R.S. 2004. Environmental and inoculums-source effects on resistance of F2 western white pine in British Columbia. Can. J. Plant Path. 26:351-357.

Hunt, R.S. 2005. Effect of plant age and length of growing season on the development of blister rust cankers in western white pine. Can. J. Plant Path. 27:453-457.

Hunt, R.S., Meagher, M.D. 1989. Incidence of blister rust on "resistant" white pine (*Pinus monticola* and *P. strobus*) in coastal British Columbia plantations. Can. J. Plant Path. 11:419-423.

Huxley, J.S. 1942. Evolution: The modern synthesis. London: Allen and Unwin.

Johannes, F., Colot, V., Jansen, R. 2008. Epigenome dynamics: a quantitative genetics perspective. Nature Reviews Genetics 9:883-890.

Johannes, F., E. Porcher, F.K. Teixeira, V. Saliba-Colombani, M. Simon, N. Agier, A. Bulski, J. Albuisson, F. Guerche, F. Hospital, V. Colot. 2009. Assessing the impact of transgenerational epigenetic variation on complex traits. PLoS Genetics 5:1-11.

Jablonka, E., Lamb, M.J. 2008. Soft inheritance: Challenging the Modern Synthesis. Genetics and Molecular Biology. 31:389-395.
Jones, J.D.G., Dangl, J.L. 2006. The plant immune system. Nature 444:323-329.
Jung, J., Seo, P., Park, C. 2009. MicroRNA biogenesis and function in higher plants. Plant Biotechnol Rep. 3:111-126.
Kattera, J., Hiltunen, R. 2010. Susceptibility of *Pedicularis* spp. To *Cronartium ribicola* and *C. flaccidum* in Finland. For. Path. In press.
Kim, M.-S., Richardson. B.A., McDonald, G.I., Klopfenstein, N.B. 2011. Genetic diversity and structure of western white pine (*Pinus monticola*) in North America: a baseline study for conservation, restoration and addressing impacts of climate change. Tree Genetics and Genomes. 7:11-21.
King. J.N., David, A., Smith, D., Smith, J. 2010. A review of genetic approaches to the management of blister rust in white pines. For. Path. 40:292-313.
Kinloch, B.B., Jr., Sniezko, R.A, Dupper, G.E. 2003. Origin and distribution of Cr2, a gene for resistance to white pine blister rust in natural populations of western white pine. Phytopathology 93:691-694.
Kinloch, B.B., Jr., Sniezko, R.A, Dupper, G.E. 2004. Virulence gene distribution of Cr2 and dynamics of white pine blister rust pathogen in western North America. Phytopathology 94:751-758.
Kinloch, B.B., Jr., Davis, D.A., Burton, B. 2008. Resistance and virulence interactions between two white pine species and blister rust in a 30-year field trial. Tree Genetics and Genomes 4:65-74.
Kliebenstein, D.J. 2010. Systems biology uncovers the foundation of natural genetic diversity. Plant Physiology 152:480-486.
Koonin, E. 2009. Darwinian evolution in the light of genomics. Nucleic Acids Research. 37:1011-1034.
Lazzaro, B.P., Little, T.J. 2009. Immunity in a variable world. Phil. Trans. R. Soc. B. 364:15-26.
Lelandais-Briere, C., Sorin, C., Declerek, M., Benslimane, A., Crespi, M., Hartmann, C. 2010. Small RNA diversity in plants and its impact in development. Current Genomics 11:14-23.
Liu, J-J., Ekramoddoullah, K.M. 2007. The CC-NBS-LRR subfamily in *Pinus monticola*: Targeted identification, gene expression, and genetic linkage with resistance to *Cronartium ribicola*. Phytopathology 97:728-736.
Liu, J-J., Ekramoddoullah, K.M. 2009. Identification and characterization of the WRKY transcription factor family in *Pinus monticola*. Genome 52:77-88.
Liu, B., Xu, C., Zhao, N., Qi, B., Kimatu, J., Pang, J. 2009. Rapid genomic changes in polyploidy wheat and related species: implications for genome evolution and genetic improvement. J. Genetics and Genomics 36:519-528.
Lu, S., Sun, Y., Amerson, H., Chiang, V. 2007. MicroRNAs in loblolly pine (*Pinus taeda*) their association with fusiform rust gall development. The Plant Journal 51:1077-1098.
Mamanova, L., Andrews, R.M., James, K.D., Sheridan, E.M., Ellis, P.D., Langford,C.F., Ost, T.W.B., Collins, J.E. 2010. FRT-seq: amplication-free, strand-specific, transcriptome sequencing. Nat Mehtods 7:130-132.
Mattick, J.S. 2009. The genetic signatures of noncoding RNAs. PLoS Genetics 5:1-12.
McDonald, G.I., Dekker-Robertson, D.I. 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, Saariselka, Finnish Forest Research Institute, Research Papers 7121:285-295.
McDonald, G.I., Hoff, R.J. 1969. Effect of rooting mediums and hormone applications on rooting of western white pine needle fascicles. USDA For. Serv. Res. Note INT-101, 6p.
McDonald, G.I., Hoff, R.J. 1970a. Effects of *Cronartium ribicola* infection on rooting potential of detached *Pinus monticola* needle bundles. Can. J. Bot. 48:1943-1945.
McDonald, G.I., Hoff, R.J. 1970b. Resistance to *Cronartium ribicola* in *Pinus monticola*: Early shedding of infected needles. USDA For. Serv. Res. Note INT-124, 8p.
McDonald, G.I., Hoff, R.J. 1971. Resistance to *Cronartium ribicola* in *Pinus monticola*: Genetic control of needle-spots-only resistance factors. Can. J. For. Res. 1:197-202.
McDonald, G.I., Hoff, R.J., Samman, S. 1991. Epidemiologic function of blister rust resistance: A system for integrated management of blister rust resistance. In: Hiratsuka, Y., ed. Rusts of Pines: Proceedings; 1989 September 18-22; Banff, Alberta, Edmonton: Forestry Canada: 235-255.
McDonald, G.I., Zambino, P.J., Klopfenstein, N.B. 2005. Naturalization of host-dependent microbes after introduction into terrestrial ecosystems. In: Lundquist, J.E. and R.C. Hamelin (ed.). Forest Pathology: From genes to Landscapes. The American Phytopathological Society, St. Paul, MN. pp 41-57.
McDonald, G.I., Zambino, P., Sniezko, R. 2004. Breeding rust-resistant five-needle pines in western United States: Lessons from the past and a look to the future. in Sniezko, R.A., Samman, S., Schlarbaum, S.E., Kriebel, H.B., eds. Breeding and genetic resources of five-needle pines: growth, adaptability, and pest resistance. IUFRO Working Party 2.02.15: proceedings: 2001 July 24-25: Medford, OR. Fort Collins, CO: USDA. Forest Service, Rocky Mountain Research Station. RMRS-P-32. pp 28-50.
Morin, R.D., Aksay, G., Dolgosheina, E.V. 2008. Comparative analysis of the small RNA transcriptomes of *Pinus contorta* and *Oryza sativa*. Genome Res. 18:517-584.
Mulvey, R.L., Hansen, E.M. 2011. Castilleja and Pedicularis confirmed as telial hosts for *Cronartium ribicola* in whitebark pine ecosystems of Oregon and Washington. For. Path. In Press.
Muro, E.M., Andrade-Navarro, M. A. 2010. Pseudogenes as an alternative source of natural antisense transcripts. BMC Evol. Biol. 10:338.
Morozova, O., Hirst, M.H., Marra, M.A. 2009. Applications of new sequencing technologies for transcriptome analysis. Annu. Rev. Genomics Hum. Genet. 10:135-151.
Nilsen, T.W., Graveley, B.R. 2010. Expansion of the eukaryotic proteome by alternative splicing. Nature 463:457-463.
Padmanabhan, C., Zhang, X., Jin, H. 2009. Host small RNAs are big contributors to plant innate immunity. Current Opinion in Plant Biology. 12:465-472.
Pandey, S.P., Somssich, I.E. 2009. The role of WRKY transcription factors in plant immunity. Plant Physiology 150:1648-1655.

Pieterse, C.M.J., Antonio Leon-Reyes, A., Van der Ent, S., Van Wees, S.C.M. 2009. Networking by small-molecule hormones in plant immunity. Nature Chemical Biology 5:308-316.
Pigliucci, M. 2010. Genotype-phenotype mapping and the end of the "genes as blueprint" metaphor. Phil Trans. R. Soc. B. 365: 557-566.
Poland, J.A., Balint-Kurti, P.J., Wisser, R.J., Pratt, R.C., Nelson, R.J. 2009. Shades of gray: the world of quantitative disease resistance. Trends in Plant Science. 14:21-29.
Raberg, L., Graham, A.L., Read, A.F. 2009. Decomposing health: tolerance and resistance to parasites in animals. Phil. Trans. R. Soc. B 364:37-49.
Richardson, B.A., Ekramoddoullah, A.K.M., Liu, J.-J., Kim, M.-S., Klopfenstein, N.B. 2010. Current and future approaches to investigate the white pine blister rust pathosystem. For. Path. 40:314-331.
Rival, A., Beule, T., Aberlenc-Bertossi, F., Tregear, J., Jaligot, E. 2010. Plant Epigenetics: From genomes to epigenomes. Not. Bot. Hort. Agrobot. Cluj 38: 9-15.
Sadd, B.M., Schmid-Hempel, P. 2009. Principles of ecological immunology. Evolutionary Applications. 2:113-121.
Schoettle, A.W., Sniezko, R.A. 2007. Proactive intervention to sustain high-elevation pine ecosystems threatened by white pine blister rust. J. For. Res. 12:327-336.
Schulenburg, H., Kurtz, J., Moret, Y., Siva-Jothy, M.T. 2009. Introduction: Ecological immunology. Phil. Trans. R. Soc. B 364:3-14.
Schwandt, J., Ferguson, B. 2003. Performance of F2 western white pine plantations in northern Idaho. In: Stone, J., Maffei, H., comp. Proceedings of the 50th Western International Disease Work Conference; October 7-11 2002; Powell River, B.C. Bend, Oregon: US Forest Service. pp. 100-105.
Schwartz, D., Robertson, H.M., Feder, J.L., Varala, K., Hudson, M.E., Ragland, G. J., Hahn, D.A., Berlocher, S.H. 2009. Sympatric ecological speciation meets pyrosequencing: sampling the transcriptome of the apple maggot *Rhagoletis pomonella*. BMC Genomics 10:633.
Siomi, H., Siomi, M.C. 2009. On the road to reading the RNA-interference code. Nature 457:396-404.
Stern, D.L., Orgogozo, V. 2009. Is genetic evolution predictable? Science 323:746-751.
Turner, B.M. 2009. Epigenetic responses to environmental change and their evolutionary implications. Phil. Trans. R. Soc. B 364:3403-3418.
Via, S. 2009. Natural selection in action during speciation. PNAS 106:9939-9946.
Wang, H., L. Waller, S. Tripathy, S. St. Martin, L. Zhou, K. Kramps, D. Tucker, Y. Mao, I. Hoeschele, M. Saghi Maroof, B. Tyler, and A. Dorrance. 2010. Analysis of genes underlying soybean quantitative trait loci conferring partial resistance to *Phytophthora sojae*. The Plant Genome 3:23-40.
Whittle, C.I., Otto, S., Johnston, M., Krochko, J. 2009. Adaptive epigenetic memory of ancestral temperature regime in *Arabidopsis thaliana*. Botany 87:650-657.
Wolf, J.B.W., Lindell, J., Backstrom, N. 2010. Speciation genetics: current status and evolving approaches. Phil. Trans. R. Soc. B 365:1717-1733.
Woo, K-S, Fins, L., McDonald, G.I., Wiese, M.W. 2001. Differences in needle morphology between blister rust resistant and susceptible white pine stocks. Can. J. For. Res. 31:1880-1886.
Woo, K-S, Fins, L., McDonald, G.I., Wenny, D.L., Eramian, A. 2002. Effects of nursery environment on needle morphology of *Pinus monticola* Dougl. and implications for tree improvement programs. New Forests 24:113-129.
Woo, K.S., McDonald, G.I., Fins, L. 2004. Influence of seedling physiology on expression of blister rust resistance in needles of western white pine. Pp 250-254 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 July 24-25: Medford, OR, USA. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station. RMRS-P-32.
Yakovlev, I.A., Fossdal, C., Johnsen, Q. 2010. MicroRNAs, the epigenetic memory and climatic adaptation in Norway spruce. New Phytologist. In press.
Zemach, A., McDaniel, I.E., Silva, P., Zilberman, D. 2010. Genome-wide evolutionary analysis of eukaryotic DNA methylation. Science 328:916-919.
Zhang, S., Sun, L., Kragler, F. 2009. The phloem-delivered RNA pool contains small noncoding RNAs and interferes with translation. Plant Physiology 150: 378-387.
Zhang, W., Gao, S., Zhou, X., Xia, J., Chellappan, P., Zhou, X., Zhang, X., Jin, H. 2010. Multiple distinct small RNAs originate from the same microRNA precursors. Genome Biology 11:r81.
Zhu, Y., Qian, W., Hua, J. 2010. Temperature modulated plant defense responses through NB-LRR Proteins. PLoS Pathogens 6:e1000844.