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HOST-PATHOGEN SPECIFICITY IN THE WHITE PINE-BLISTER RUST PATHOSYSTEM.

Frank Edward Williams

M.P.M., Simon Fraser University, 1982

B.Sc., University of Victoria, 1980

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Host-pathogen specificity in the white pine blister rust pathosystem.

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ABSTRACT

One system of classifying plant resistance to parasites is to divide all resistance occurring between a single host species and a single parasite species into two types; vertical and horizontal. Vertical resistance is based on the presence of a gene-for-gene relationship between the host and parasite, and so tends to be specific resistance, where a single host individual may be highly resistant to some parasite individuals, but not to others. Horizontal resistance is that which does not involve a gene-for-gene relationship, and tends to be nonspecific, so that a given host individual is resistant to virtually all individuals of a given parasite species. Many gene-for-gene relationships have been demonstrated in pathosystems which involve domesticated plants, but no undomesticated plant has been shown to be part of a gene-for-gene relationship.

Most gene-for-gene relationships have been demonstrated by simultaneous genetic analysis of both host and parasite, but such relationships may also be demonstrated by analysis of host-parasite interactions. The latter technique was chosen for this thesis, and was applied to the Ribes bracteosum - Cronartium ribicola pathosystem. Ribes species are alternate hosts for Cronartium ribicola, the causal agent of white pine blister rust, an introduced stem rust of five-needle pines. The results show that a gene-for-gene relationship is present in the R. bracteosum

- C. ribicola pathosystem, thus indicating that vertical resistance is not an artifact of agriculture, and may be expected to occur in wild pathosystems.

Single spore derived isolates of C. ribicola were also used to inoculate detached needles of Pinus monticola. The results of these experiments suggest that specific resistance may be present in the P. monticola - C. ribicola pathosystem.

Imagination is more important than knowledge.

Albert Einstein

There is something fascinating about science. One gets such a wholesale return of conjecture out of a trifling investment of fact.

S. Clemens

All time is game time.

Rules. All games.
NATO Studies Centre
London

About field research: "If I knew what we'd find, I wouldn't bother to find it. People think research is like cutting wood and stacking it up. I was working with Cap'n Cousteau. We worked and we worked, didn't get anywhere. That's how you know you're doing research."

Dr. H. E. Edgerton
National Geographic,
October 1987.

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Chapter 1.

1.1 Introduction.

Plant diseases have likely been recognized and described since the beginning of agriculture, if not before. Perhaps the earliest record of variation in the levels of plant disease is the third century writings of Theophrastus, who noted that barley was more liable to mildew than was wheat, and that among barleys some were mildewed more than others (Carefoot and Sprott 1967). Consideration of plant disease remained largely descriptive however, until the recognition of Mendel's laws of inheritance, at the end of the nineteenth century.

Mendel's work with garden peas was the start of the science of genetics, and much of his terminology (hybrid, dominant, recessive) is still in use today. That his work and its implications went unrecognized for nearly forty years is a major tragedy; fortunately it was not lost. Mendel's work was recognized independently by three scientists around 1900 (Kirk 1975). At this time Mendel's conclusions were compatible with current biological theory, and within a decade there were more than 200 reports of breeding studies of both plants and animals. These studies formed the basis of modern plant breeding.

The variation in disease levels on different hosts has

continued to intrigue crop scientists, and succeeding studies showed that such differences could occur at various taxonomic levels. That host species are subject to diseases which have no effect on other species has been a common observation, as was the fact that host species and populations appeared to vary in the amount of disease shown. Biffen (1905) showed that resistance in wheat to yellow rust (Puccinia striiformis) was governed by a single recessive gene, thus demonstrating that host resistance had a genetic basis. Variation in pathogenicity within a pathogen species was first reported by Erikson (1894), who, by using different host species, subdivided Puccinia graminis into formae speciales.

Barrus (1911) demonstrated the existence of physiological races in Colletotrichum lindemuthianum, and pathogenic variation within a single host species. Goldschmidt (1928) studied the genetics of pathogenicity in the rust Ustilago violacea, and discovered that the pathogenicity of the physiologic races was controlled by single genes, in much the same way as resistance was controlled by single genes in the host.

From these early results it could be seen that much of the variation seen in both host and parasite obeyed Mendel's laws. Our understanding of plant resistance to parasites reached another milestone with the discovery (Flor 1942) of the genetic interaction which is the basis of at least some host-parasite relationships. Working with the flax-Melampsora lini system, Flor demonstrated that single gene resistance in the host became

susceptibility when the resistance gene or genes were matched by pathogen virulence genes. This system of matching genes in host and parasite has become known as the gene-for-gene relationship, and it is one of the cornerstones of modern plant breeding. The gene-for-gene relationship concept has been broadened to include parasites other than plant pathogens, and the list of plant parasites for which gene-for-gene relationships have been demonstrated includes insects, viruses, nematodes, and bacteria. Gene-for-gene relationships have been conclusively demonstrated to occur in relatively few host-parasite associations, but are suspected to occur in many more.

Frequently, host resistance does not appear to be determined by gene-for-gene relationships. Such resistance is called horizontal resistance in this thesis, and is discussed more fully in section 2.2.

Until now, gene-for-gene relationships have been demonstrated only with domesticated angiosperm hosts. This has lead several authors to suggest that such relationships may be an artifact of plant domestication and breeding (Vanderplank 1975). Others, such as Person (1959) have pointed out that a resistance gene of major effect would confer a large selective advantage to those individuals which possess it, relative to those which do not. This selective advantage would ensure that such genes would be maintained in natural host populations, should they occur there. Eventually this would lead to the evolution of a gene-for-gene relationship. By

examining a white pine-blister rust pathosystem, it was hoped to determine whether gene-for-gene relationships occur in the wild, with wild hosts, whether such relationships could occur with a gymnosperm host, and whether both host species of a heterecious parasite could have a gene-for-gene relationship with their common parasite.

There were several reasons for choosing the white pine-blister rust association for this study. The first gene-for-gene relationship demonstrated was in a rust-host pathosystem (Flor 1942), and since then, several more rust-host pathosystems have been shown to include such relationships (Day 1974). Thus if gene-for-gene relationships exist in natural pathosystems, they are as likely to exist in rust-host pathosystems as in any other. Secondly, white pine blister rust is economically important, and because of this there has been as much research effort devoted to its study as there has been for any wild host-parasite association. Consequently, much scientific literature is available.

White pines, of which Pinus monticola Dougl. is the most abundant in the Pacific Northwest, are among the most economically desirable trees in North America. White pines have wide site tolerances and are able to grow well on sites which do not support other commercial species (Allen 1959). White pines are resistant to root rot (Phellinus weirii (Murr.) Gilbertson) (Allen 1959), and the lumber is more valuable than either Douglas fir (Pseudotsuga menziesii (Mirb.) Franco), lodgepole pine (Pinus

contorta Dougl.), or hemlock (*Tsuga heterophylla* (Raf.) Sarg.), which are the species most commonly planted on potential white pine sites (B.C. Ministry of Forests 1979). On most sites, *Pinus monticola* grows faster than do other conifers, thus producing a greater volume of wood in a given time period (Deitschman and Green 1965; Packee 1976). Thus any research which contributes to the reestablishment of western white pine would be both scientifically and economically valuable.

The remainder of Chapter 1 is concerned with an introduction to wild pathosystems, and a summary of the white pine-blister rust pathosystem. The understanding of this thesis rests upon an understanding of the gene-for-gene relationship; therefore Chapter 2 is a review, and interpretation of this relationship. Chapter 3 is devoted to examination of the *Ribes*-*Cronartium ribicola* pathosystem, while Chapter 4 deals with the *Pinus*-*C. ribicola* pathosystem.

1.2 Wild plant pathosystems.

A plant pathosystem is defined as a host-parasite complex in which the host is a population of a single plant species and the parasite is a population of a single species. Robinson (1987) has identified three categories of plant pathosystems; crop pathosytems, wild pathosystems, and weed pathosystems. Only crop pathosystems and wild pathosystems are relevant to this thesis.

A wild plant pathosystem is one in which human involvement has been minimal and has not affected the selection pressures acting on either the host or parasite. Crop pathosystems differ from wild plant pathosytems by having a host which has been domesticated. Domestication invariably results in the application of selection pressures which are very different from natural selection pressures, and this can be expected to affect the character of both host and parasite. Consequently the nature of the resistance/ susceptibility relationships may be quite different in each of the two types of pathosystem.

Comparatively little information concerning host-parasite interactions in wild plant pathosystems has been collected, and so our knowledge of their nature is limited. There has been a tendency to assume that wild plant pathosystems behave similarly to crop pathosystems.

It is generally accepted that the level of parasitism in wild plant pathosystems is very low (Dinoor and Eshed 1984). This is held to be due to the diversity of plants in the wild, this diversity being both between and within plant species. Crop pathosystems differ fundamentally and have host populations which are genetically uniform or very similar. However, little is known about the nature of host resistance in wild plant host species, and natural stands of very few species (e.g. coniferous forests, grassland steppes, and tropical savannas) which cover large areas may closely resemble crop pathosystems. Yet failures of resistance of the kind associated with the "boom and bust" cycles of crop pathosystems have not been documented in wild plant pathosystems. Thus it would be valuable to examine the evidence for variability of resistance in wild plant pathosystems, at the different levels at which it may occur.

Variation in resistance between different wild host species to a single parasite has been demonstrated by numerous authors (Figoni et al. 1983; Mielke et al. 1937). Variation in resistance between species may be expected to be the rule, for different species vary in a multitude of ways, and it is likely that at least some of these differences will affect susceptibility. Such "non-host" resistance is considered to be outside the conceptual boundaries of the pathosystem, and outside the gene-for-gene relationship.

Variation in resistance within a wild species is also to be expected, on the grounds that there can be no selection

without variation, and that variation has been shown to occur in most of the wild plant hosts studied. What needs to be done now is to determine the character of such resistance as is found. Is it general resistance or specific? Horizontal resistance or vertical resistance? The most important question to be answered is whether gene-for-gene relationships are present in wild pathosystems. If such relationships are present in wild pathosystems, the host population will have to be managed so as to minimize the possibility of a "bust".

The gene-for-gene relationship has become one of the cornerstones of our understanding of plant host-parasite interactions, yet all demonstrations of gene-for-gene relationships have been made in crop pathosystems. No undomesticated host species has been shown to have a gene-for-gene relationship with any of its parasite species. This can be interpreted as being due either to the far greater research emphasis on domesticated plant species, or as negative evidence which suggests that such relationships do not exist in wild pathosystems. The presence or absence of gene-for-gene relationships in wild pathosystems is of great interest to theoretical plant pathology, and is of great importance to the managers of undomesticated plant resources.

Although research emphasis has been largely concerned with more economically important crop pathosystems, wild pathosystems have received some investigation. Some of these investigations, such as the examination of a rust disease of wild sunflowers

(Zimmer and Rehder 1976), Puccinia spp. on Avena spp. (Burdon et al. 1983; Dinoor 1977; Wahl et al. 1978), and Erysiphe graminis hordei on Hordeum spp. (Wahl et al. 1978), were compromised by the presence of cultivated relatives nearby. Because these relatives are also susceptible to the pathogens of the wild plants, and may have some pathogen and genetic exchange with the wild hosts, it would be dangerous to assume that the pathosystems of these studies are representative of wild pathosystems. Furthermore, the investigation of possible gene-for-gene relationships was not a primary objective of these studies. In most cases, the pathosystem was not investigated at the intra-specific level, and consequently evidence for gene-for-gene relationships, if present, would not have been seen.

Deciduous forest species frequently fit the criteria for wild pathosystems, and have been subjected to study aimed at detection and characterization of intraspecific resistance in the host. The Melampsora occidentalis - Populus trichocarpa pathosystem has been shown to exhibit evidence of quantitative resistance, but revealed no evidence of qualitative resistance (Hsiang and van der Kamp 1985). Populus deltoides clones have been shown to vary qualitatively in their response to four mono-uredospore isolates of Melampsora larici-populina, with a significant interaction between clones and isolates (Chandrashekar and Heather 1980). These results are highly suggestive of specific resistance, but insufficient data was

presented to allow demonstration of a gene-for-gene relationship. The data requirements for a demonstration of a gene-for-gene relationship is discussed in detail in Chapter 2.

The Ribes-Cronartium ribicola pathosystem has also been investigated with the aim of characterization of host resistance. A summary of the results of these investigations is given in section 3.1.

Conifer forest species have also been investigated for their resistance to parasites. Pinus taeda families have been shown to vary in their response to different sources of Cronartium fusiforme inoculum (Powers et al. 1977; Powers and Zobel 1978; Powers, 1980), but the variation was suggestive of non-specific resistance. Kinloch and Littlefield (1977) demonstrated the existence of a single major gene for resistance to Cronartium ribicola in Pinus lambertiana (sugar pine), and the presence of a matching race of pathogen. Their results can be used to construct a Loegering and Powers (1962) quadratic check. To date, the work of Kinloch and Littlefield (1977) is the strongest evidence of specific, monogenic resistance in a wild pathosystem. Such resistance is suggestive of, but does not necessarily indicate, the presence of a gene-for-gene relationship (see 2.5).

Because Cronartium ribicola is considered to be a relative newcomer to the Pacific north-west, it is important to note that the local pathosystems may not be representative of more established wild pathosystems. This may be less of a concern for

the Ribes-blister rust pathosystem than it is for the pine-blister rust pathosystem, as the Ribes host has a much faster reproductive cycle, and so should be more responsive to new selection pressures.

1.3 The white pine-blister rust life cycle.

The causal agent of white pine-blister rust is Cronartium ribicola J. C. Fisch. ex Rabenh., a heteroecious fungus of the order Uredinales (rusts). The host range of C. ribicola includes all five-needle pines (also known as 'white' pines, or 'soft' pines) as hosts for the sexual recombination phase, and any species of Ribes (wild or domestic currants) for the asexual reproduction phase. Castilleia miniata can also serve as an alternate host (Hiratsuka and Maruyama 1976), as can Pedicularis resupinata (Yi and Kim 1983). There are eight species of white pine (P. monticola, P. strobus, P. lambertiana, P. albicaulis, P. flexilis, P. aristata, P. cembroides, and P. balfouriana) native to North America, and 30 species of wild Ribes can be found in the Pacific Northwest (Hitchcock and Cronquist 1974). There is evidence which suggests that there are two formae speciales of the white pine blister rust fungus (Patton and Spear 1989), one originating in North America (C. ribicola f. sp. ribicola), and the other (C. ribicola f.sp. pedicularis) from East Asia.

C. ribicola is native to Asia. From here it spread to Europe in the 19th century, and spread from Europe to North America in the early 1900's (Littlefield 1981). In both Europe and North America the fungus quickly reached epidemic proportions, and has extended its range to match the natural

distribution of its hosts. White pine-blister rust is one of the most important forest diseases in North America, and is a major forestry problem wherever white pines and Ribes coexist. It is also a problem for producers of cultivated currants, as many commercial varieties of black currants (R. nigrum) have proved highly susceptible to infection by the fungus (Hahn 1948). Fortunately, resistant black currant cultivars have been developed (Anderson and French 1955).

Infection of the pine host begins when a germ tube of a haploid basidiospore enters a stomate of a white pine needle, and successfully penetrates the spongy mesophyll layer (Clinton and McCormick 1919; Patton and Johnson 1970). The fungus grows through the needle and enters the needle fascicle. From here it grows into the twig, branch, or stem to which the fascicle is attached. Here the fungus colonizes and disrupts the vascular tissue, eventually girdling it, and so killing itself and that part of the tree which is distal to the girdle. When this is a twig, the loss to the tree is minor; when it is a branch, the loss is more serious; when it is the stem of the tree below the crown, the tree dies.

The period from infection to girdling is known to depend upon the diameter of the stem being girdled, with larger stems taking longer to girdle than smaller stems (Ziller 1974). It is suspected to vary between trees, and between fungus isolates. Aeciospore production usually requires 2 or more years post infection (Hunt 1983), so many twig infections may be considered

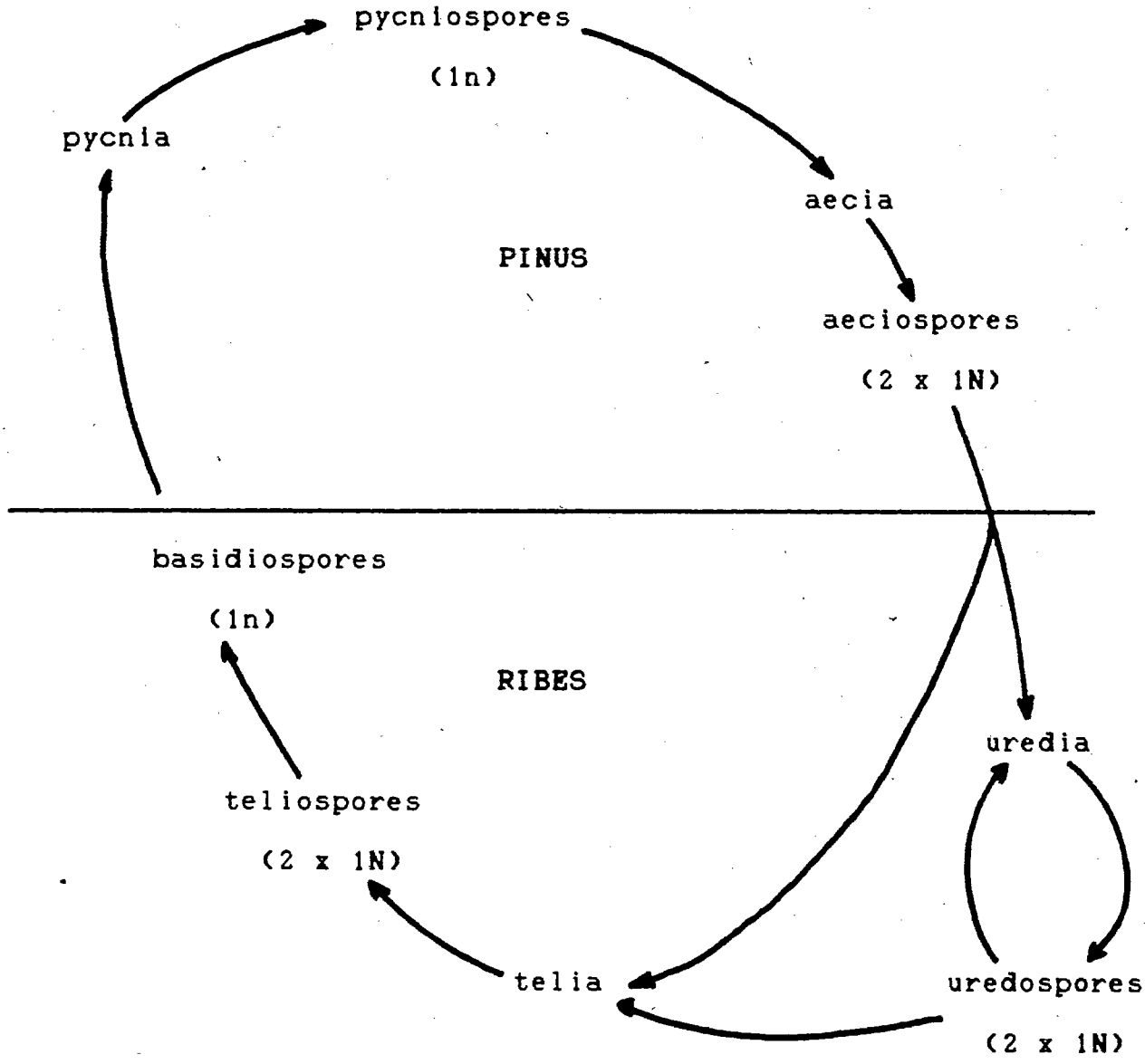
to be unsuccessful from the fungal point of view, as twig girdling frequently takes place before sporulation can occur. Branch and stem infections may sporulate for years, and prior to complete girdling, infections appear to have little effect on tree growth (Hoff 1984).

Aeciospore production of the fungus on the pine host generally takes place during the second or third spring after infection (Hunt 1983). There is strong evidence that the rust is heterothallic, and that aeciospore production requires insect vectors for cross fertilization (Hunt 1985). The spores are produced in aecia, which form the distinctive blisters from which the name of the disease is derived. Unlike the basidiospores which infect the pine, the aeciospores are capable of long distance air-borne dispersal, being resistant to both desiccation and ultra-violet light. C. ribicola aeciospores can be wind transported many kilometers, and remain viable for many weeks (Mielke 1943). The aeciospores cannot infect pine trees, but must travel to the alternate host, the Ribes plants. Here, infection occurs through the stomata, and usually on the underside of the leaf. Possibly to facilitate this, aeciospores are attracted to negatively charged objects (personal observation), presumably because they carry a partial positive charge. This would facilitate sticking to leaf tissue, which carries a partial negative charge. Infection requires free water be present at the infection court, and is most successful when accompanied by periods of darkness and temperatures between 13-18

C (Van Arsdel et al. 1956). Infection fails to occur at temperatures above 23 C (Appendix 1).

Within 2 - 3 weeks post infection, depending largely on temperature, aeciospore infections produce uredospores, which can infect more Ribes tissue. Like aeciospores, uredospores are capable of long distance dispersal, but in practice most uredospores probably re-infect the host on which they have been produced (Hunt 1983). This polycyclic phase is repeated until mid-summer, when both temperature cues (McDonald and Andrews 1980) and leaf senescence (Williams unpublished) stimulate the Ribes infections to produce telia rather than uredospores. The telia consist of teliospores, which germinate to produce haploid basidiospores. The basidiospores are sensitive to both desiccation and ultra-violet light, and so are short lived. Consequently they are only capable of short distance dispersal of little more than a few hundred metres under most conditions, but have been shown to be able to be able to infect pines at ranges of several kilometers under optimum conditions (Van Arsdel 1967). The basidiospores complete C. ribicola's life cycle by infecting the pine host. Successful infection of the pine requires high humidity, free water, temperatures of 10-13 C, and darkness (Van Arsdel et al. 1956). In the field, basidiospores appear to be released mainly at night, when conditions are most favorable (Van Arsdel 1967). A diagrammatic description of C. ribicola's life cycle is shown in figure 1.1.

Figure 1.1 The white pine-blister rust life cycle.



Chapter 2. The gene-for-gene relationship.

2.1 Introduction.

Although the concept of the gene-for-gene relationship has transformed the understanding of host-parasite interactions, the number of proven gene-for-gene relationships remains small, and has been limited mainly by the time-consuming tests which are required for a genetic demonstration. Analysis of host-parasite differential interactions provides a more rapid, phenotypic demonstration of a gene-for-gene relationship. This chapter describes the host-parasite differential interactions necessary for phenotypic demonstration of gene-for-gene relationships, and includes a list of gene-for-gene relationships which have been identified phenotypically.

2.1 Review of the gene-for-gene hypothesis.

Flor's (1942; 1946; 1947) gene-for-gene hypothesis states that for every Mendelian gene for resistance in the host plant species there is a corresponding and specific gene for virulence in the pathogen species. When host resistance genes are matched by parasite virulence genes, the resistance does not operate. When host resistance genes remain unmatched, the resistance operates. Thus "each gene in either member of a host-parasite

system may be identified only by its counterpart in the other member of the system" (Flor 1971). Robinson (1987) has compared this relationship to that which occurs between locks and keys; a key must match a lock before it can turn the lock, and each can be used to identify the other.

Flor's (1942; 1946) gene-for-gene hypothesis was the result of simultaneous genetic investigation of a host plant (flax - Linum usitatissimum) and its pathogen (flax rust - Melampsora lini). In this host-pathogen association, resistance has usually been shown to be a Mendelian dominant, and virulence is usually a Mendelian recessive. The gene-for-gene hypothesis does not require that resistance be dominant, or virulence be recessive, but this has been the most frequently demonstrated situation. Vanderplank (1984) points out that such situations are more easily identified than those which involve incomplete dominance or additive gene action, and this may account for the apparent lower frequency of the more complex interactions. When a resistance gene is expressed, it usually provides near total protection against all parasite races which do not have the corresponding virulence gene(s).

The important implication for the plant breeder is that where resistance is part of a gene-for-gene relationship, it will remain effective only as long as those parasite races with the matching virulence genes are rare or absent from the pathosystem. Since the widespread use of such resistance can be expected to provide a powerful selective advantage to those races which can

match it, such resistance can be predicted to be temporary. Thus the "boom and bust" cycle which has accompanied many crops using gene-for-gene based resistance. For the plant breeder, it is crucially important to determine whether the resistance being observed is part of a gene-for-gene relationship.

It is not always appreciated that a gene-for-gene relationship (Flor 1942; Person 1959) can be demonstrated without resorting to complex genetic investigations. An alternative method (Person 1959) involves the analysis of the differential interactions between hosts and parasites, in much the same way as antigens and antibodies can be identified by their interactions (Person 1959; Person and Christ 1983). There are, however, various categories of differential interaction matrices, and only some of them can identify a gene-for-gene relationship. This chapter identifies those kinds of matrices which can provide a demonstration of a gene-for-gene relationship, and illustrates how these matrices lead to a demonstration of a gene-for-gene relationship.

In its original form (Flor 1942; Person 1959) the gene-for-gene relationship was applied to host-pathogen systems. The concept has since been broadened to include other parasites in addition to plant pathogens. Gene-for-gene relationships have been demonstrated in host-fungus systems, in a host-insect system (Hatchett and Gallun 1970), in a host-virus system (Drijfhout 1978), and has been suggested to occur in a host-nematode system (Jones and Parrot 1965), and in a bacterium-host system (Mew et

al. 1982). For the rest of this paper, the term 'parasite' is used in the sense of 'host-parasite' association, to include plant pathogens and other kinds of plant parasites.

Gene-for-gene relationship theory has been further broadened to include one-gene-for-more-than-one-gene relationships. A one-gene-for-more-than-one-gene relationship has been demonstrated by Christ and Groth (1982), and although the existence of such relationships has considerable genetic significance, they have little phenotypic or pathosystem effect. A one-gene-for-more-than-one-gene relationship may be seen as a special kind of gene-for-gene relationship, and although its existence is acknowledged, it does not require special distinction in this chapter.

When a gene-for-gene relationship is present, an individual host normally possesses a subset of the set of resistance genes available in the host population, and an individual parasite normally possesses a subset of the set of virulence genes present in the parasite population. When the host resistance genes are matched by the parasite virulence genes, and conditions are suitable, infection is successful and the host is described as susceptible. When one or more of the host resistance genes remains unmatched, the infection fails, and the host is described as resistant. The gene-for-gene relationship defines the concepts of both vertical resistance (Vanderplank 1963; 1968) and the concept of the vertical subsystem of a pathosystem (Robinson 1976; 1980). Vertical

resistance in crops is often ephemeral, is usually qualitative in its effects, and is race-specific.

The absence of a gene-for-gene relationship normally results in a continuous range of host-parasite interactions (constant ranking; Fig. 2.1); this is the definitive characteristic of horizontal resistance (Vanderplank 1963; 1968), and the horizontal subsystem of a pathosystem (Robinson 1976; 1980). Horizontal resistance in crops is associated with durability, is usually quantitatively inherited, and is race-nonspecific.

Johnson (1983) has pointed out that durable resistance (*sensu* Scott *et al.*) cannot be predicted: it can only be deduced by the test of time. It is impossible to prove the negative (i.e. resistance will not fail) but it is possible to prove the positive (i.e. resistance will fail). Race-specific resistance cannot be positively identified in the absence of races which possess matching genes for virulence, and only field testing over large areas for a reasonable time period can make it likely that the resistance in question has been exposed to all possible pathotypes (Johnson 1983). Demonstration of a gene-for-gene relationship provides conclusive evidence that the resistance in question is likely to fail, since it proves the existence of matching pathotypes. When matching pathotypes are epidemiologically competent (i.e. have a progeny/parent ratio greater than one), resistance will fail. Predictability of resistance

failure is essential for an effective disease control strategy. It is particularly important in a long-life perennial crop such as plantation forests.

Traditionally, the demonstration of a gene-for-gene relationship has required detailed genetic analyses in both the host and the parasite. When the sexual phase of the parasite is technically unusable (as with aphids) or absent (as with viruses, bacteria, and imperfect fungi), such genetic studies are at best difficult, and at worst impossible. Consequently alternative methods of demonstrating gene-for-gene relationships are valuable, and this value increases with increasing ease of demonstration.

2.3 Differential interactions.

An important characteristic of a gene-for-gene relationship is the presence of one or more differential interactions between subpopulations of the host species (cultivars, varieties or clones) and subpopulations of the parasite species (races, strains, isolates, or biotypes). Where subpopulations are defined by the presence or absence of resistance genes in the host, and virulence genes in the parasite, special terminology is useful. Robinson (1976; 1979) has suggested that resistance gene defined subpopulations of the host (biotypes grouped together on the basis of their possession of the same resistance genes) be

called vertical pathodemes, and that virulence gene defined subpopulations of the parasite be called vertical pathotypes. This terminology is used in this thesis.

A differential interaction may be defined as two different parasitic interactions, one resistant and one susceptible. Where qualitative differences are not obvious (as is the case with most quantitative data), analysis of variance tests may be used to determine the validity of a suspected differential interaction (Vanderplank 1975; Scott et al. 1980), as may ranking tests (Vanderplank 1975; 1984). Without significant differences in host susceptibility it is not possible to demonstrate a differential interaction.

In practice, the pathodemes most commonly used are genetically uniform cultivars (such as pure lines, clones, or hybrid varieties), and each pathotype consists of a single isolate from a population of the parasite. A differential interaction is the phenotypic expression of host susceptibility or resistance, and parasite virulence or avirulence; it is thus a pair of observations, one showing a relatively high level of disease, the other showing absence (or minimal presence) of disease. A set of differential interactions is obtained when two or more different pathotypes are used to inoculate two or more different pathodemes.

Some differential interactions may be due to causes other than a gene-for-gene relationship (i.e. all gene-for-gene relationships exhibit differential interactions, but not all

differential interactions are due to a gene-for-gene relationship).

To demonstrate a gene-for-gene relationship conclusively by means of differential interactions, it is essential to eliminate the possibility that a given set of differential interactions is due to any other cause. To do this it is necessary to review the other causes of differential interactions. Robinson (1979) lists nine alternative causes of a differential interaction.

2.4 Categories of differential interaction.

Several types of differential interaction may occur when more than one host species and more than one parasite species are used to generate differential interactions (DI). These are the qualitative polyphyletic DI, the quantitative polyphyletic DI, the hybridizing host DI, and the immunity DI. These DI's involve more than one host species, and more than one parasite species. The gene-for-gene relationship is restricted to parasitic associations which involve a single host species and a single parasite species. Consequently differential interactions in which either the pathodemes or the pathotypes involve more than one species cannot be used to demonstrate a gene-for-gene relationship.

In some parasitic associations, particularly those which

extend over a large geographic range, a differential interaction due to local differences in the levels of horizontal resistance and parasite aggressiveness (sensu Vanderplank 1975) could occur. It is possible that such local differences, combined with co-adaptation of local host and parasite isolates could be sufficiently pronounced to give a differential interaction that could be confused with a differential interaction caused by quantitative vertical (gene-for-gene based) resistance. Jeffrey et al. (1962), Jinks and Grindle (1963), and Caten (1974), report quantitative data which show such differential interactions in the potato-Phytophthora infestans pathosystem. These results appear to describe horizontal resistance which is combined with non-genetic, reversible parasite adaptation to the host, similar to adaptation to different growth media. The low level specificity causes a small deviation from constant ranking. Judging from their frequency in the literature, such differential interactions are rare. Moreover, they do not appear to affect the durability of horizontal resistance. For this chapter, such deviations from the definition of horizontal resistance will be treated as exceptions to the rule.

It is now proposed that differential interactions of this type be referred to as Jeffrey differential interactions. Jeffrey DIs are produced by small but consistent (and therefore statistically significant) differences in otherwise quantitative data. Such differential interactions do not

reflect the presence of a gene-for-gene relationship, as there appears is no specific matching between host and parasite. The possibility of a Jeffrey DI being confused with a differential interaction caused by a gene-for-gene relationship can be precluded by the use of qualitative data to derive differential interactions (since qualitatively different data are an indicator of major gene effects), Mendelian inheritance ratios, or the shape of certain differential interaction matrices.

Differential interactions may occur when the mechanisms of resistance or virulence are greatly affected by environmental factors. Such interactions are commonly referred to as environmental differential interactions, and may be expected to occur where environmental factors can determine whether infection will be successful. Temperature sensitivity of resistance genes is a well researched example of a situation which may produce an environmental differential interaction, as is site specificity. When an environmental DI occurs, susceptibility and resistance will be correlated with certain environmental factors, such as temperature, or site characteristics.

If the possibility of an environmental DI being confused with a gene-for-gene relationship is to be completely removed, each set of differential interactions must be evaluated in a single environment, at one time. Fortunately, this is the usual means of generating such differential interactions and so

presents no serious problems. A demonstration of Mendelian inheritance of resistance and pathogenicity will also remove the possibility of an environmental DI being confused with a gene-for-gene relationship, as will replicated experiments, and matrices larger than a 2 pathodeme x 2 pathotype interaction.

A resistance gene in a gene-for-gene relationship provides host resistance against pathotypes which do not have the matching virulence gene. A differential interaction may reflect a host gene with alleles conferring resistance or susceptibility to a toxin produced by a pathogen. Such differential interactions can be shown for a number of diseases, such as Victoria blight on oats, Milo disease on sorghum, and Southern leaf blight on maize (Vanderplank 1978). Differential interactions of this type are not considered to be part of a gene-for-gene relationship as there is no evidence that there is any matching of alleles conferring resistance and virulence. Flor's (1942) gene-for-gene hypothesis states that for each resistance gene there exists a specific and related virulence gene. This is the same interpretation used by Person (1959). Thus gene-for-gene relationships are defined by matching host resistance genes and parasite virulence genes, not host susceptibility genes and parasite avirulence genes, which do not match. Consequently, differential interactions which reflect the presence of a gene for susceptibility (for which no pathotype is avirulent) cannot be used to demonstrate a gene-for-gene relationship. It may

be the 'universal susceptible' pathodeme, but more information than a susceptibility DI is necessary to determine this.

The 'quadratic check' of Loegering and Powers (1962) shown in Fig. 2.2a depicts a matrix of interaction phenotypes which may result from a gene-for-gene relationship. When a gene-for-gene relationship is involved, this matrix results from the interaction of the two parasite phenotypes (virulent and avirulent) and the two host phenotypes (resistant and susceptible) which are due to a single pair (one host, one parasite) of matching genes. It does not however, provide a conclusive demonstration of a gene-for-gene relationship (Day 1974; Vanderplank 1978); environmental DIs can produce a Loegering and Powers quadratic check, as could a misinterpretation of norizontal resistance.

Person (1959) first showed that a non-genetic demonstration of a gene-for-gene relationship is possible using a model DI matrix with five pairs of matching genes. This model is now known as the 'Person differential interaction matrix' and it has a 25 x 25 (i.e. 32 x 32) matrix (Fig. 2.3). The DIs in both the host (H) and the parasite (P) are arranged according to the binomial distribution and there is a mirror image symmetry on each side of the diagonal from bottom-left to top-right. As Person (1959) indicated, when a set of experimental DIs can be arranged to fit the Person DI matrix, this will provide a conclusive demonstration of a gene-for-gene relationship.

Bettencourt and Noronha-Wagner (1967) were the first to utilize the Person DI matrix in this way, demonstrating a gene-for-gene relationship for coffee (Coffea arabica) and coffee leaf rust (Hemileia vastatrix), in which the sexual phase is unknown.

Robinson (1976; 1980) produced a more symmetrical version of the Person DI matrix by arranging the differential interactions in both the host and the parasite in the numerical order of their Habgood (1970) names, modified by Robinson (1976), and this is known as the Person/Habgood DI matrix (Fig. 2.4). The Habgood nomenclature uses binomial numbers (20, 21, 22, 23 etc.) with arithmetic values 1, 2, 4, 8, etc. to designate individual resistance and virulence genes. Each pair of matching genes in the gene-for-gene relationship is given a unique binomial number. The Habgood name of any particular phenotype is the value represented by the sum of the arithmetic values of all the genes present in that genotype. Thus Habgood '7' consists of vertical genes 1, 2, and 4. The Person/Habgood DI matrix was used by Robinson (1979) to demonstrate the presence of a gene-for-gene relationship between Piricularia oryzae and rice (Oryza sativa).

Examination of the Person/Habgood DI matrix reveals that its matrix is a system of quadratic checks (Fig. 2.4), and that these are organized into 4 x 4 matrices, each of which contains 3 quadratic checks. Each 4 x 4 matrix represents two pairs of genes, with four different resistances and four different virulences. The problem addressed in this chapter

concerns the minimum size of the matrix necessary for a conclusive phenotypic demonstration of a gene-for-gene relationship. The Person/Habgood DI matrix provides such a demonstration but the quadratic check does not. What then is the smallest matrix which provides a conclusive phenotypic demonstration of a gene-for-gene relationship, and what is its shape?

2.5 2 x 2 matrices

The smallest number of interacting genotypes which will provide a DI matrix is four, with two host phenotypes and two parasite phenotypes; this is a 2 x 2 matrix. Various kinds of 2 x 2 matrices are possible.

The simplest 2 x 2 matrix is the Loegering and Powers (1962) quadratic check shown in Fig. 2.2a. When this matrix is the reflection of genetic differences, it is due to one pair of loci (or genes where genetic information is lacking) where resistance is expressed (by the absence or relative absence of infection) in only one host-parasite interaction of the four interactions shown in the matrix. Day (1974) and Vanderplank (1978) noted that a quadratic check does not exclude the possibility of nonspecific resistance. Various

non-genetic factors (fungicides, antibiotics) can also be the cause of a quadratic check, as can differing levels of horizontal resistance (Day 1974). Consequently a quadratic check which is not supported by evidence of monogenic inheritance cannot be considered as evidence of a gene-for-gene relationship.

Demonstration of monogenic inheritance of resistance and virulence precludes the possibility of non-genetic factors as the basis for the quadratic check in question, but does not entirely rule out the possibility that the matrix is caused by horizontal resistance. For example, if a disease ranking of ≥ 1 was interpreted as susceptibility, and a disease ranking of < 1 was interpreted as resistance, the bottom right hand corner of figure 2.1 (constant ranking) could be interpreted as a quadratic check.

Vanderplank (1978) has noted that parasitic associations in which different amounts of a pathotoxin are coded for by different pathogen genes, and different levels of tolerance were coded for by different host genes, could result in a quadratic check which would not be due to a gene-for-gene relationship. An even simpler hypothesis would be the presence of oligogenically inherited horizontal resistance (Robinson 1976), or the presence of a susceptibility gene.

Essentially, a quadratic check provides no evidence of any matching of resistance and virulence genes. Because of this, a Loegering and Powers quadratic check cannot be more than

circumstantial evidence for the presence of a gene-for-gene relationship, even when host and parasite genes involved in the matrix have been identified as single genes.

In this thesis, the term "quadratic check" will only be used to describe the Loegering and Powers matrix. All other matrices should be described by their size; thus 2 x 2 matrices, 3 x 3 matrices, and so on.

A second type of 2 x 2 matrix is due to the presence of a susceptibility gene, rather than a resistance gene. A susceptibility gene requires no matching process for infection to take place, and so is susceptible to all pathotypes. This matrix is shown in Fig. 2.2b. A 2 x 2 matrix of this type can be shown for corn (Zea mais) with Texas cytoplasmic male sterility and Helminthosporium maydis, and for oats and Helminthosporium victoriae. This matrix is due to an abnormal sensitivity to a mycotoxin, or the absence of resistance genes in one host isolate; as such it is not evidence of a gene-for-gene relationship sensu Flor (1942) or Person (1959). This matrix is the converse of the quadratic check, and because it looks somewhat similar, it can lead to confusion. A 2 x 2 matrix of this type cannot be used to demonstrate a gene-for-gene relationship, as it provides no evidence of gene matching.

A third category of 2 x 2 matrix is shown in figure 2.2c. This matrix is characteristic of the 'reversed reaction type characteristic of vertical resistance' noted by Vanderplank

(1984). The matrix shown in figure 2.2c shows specificity between host and parasite; the matrices discussed above do not. Matrix 2.2c shows specificity because each isolate can infect only one of the two hosts, thus demonstrating that each host is potentially susceptible, and that each parasite is potentially virulent. To distinguish this type of matrix from the others, the term 'transagonal matrix' is proposed.

This 2 x 2 matrix could result from a misinterpretation of a polyphyletic DI or immunity DI (Robinson 1979). Under unusual circumstances, such a matrix could be the result of a complex environmental DI. If the matrix is derived from quantitative data, the matrix may be caused by a Jeffrey DI. If these possibilities can be disproved, the only hypothesis which fits the matrix is the gene-for-gene relationship.

The possibility of a matrix of this type resulting from either a qualitative polyphyletic or an immunity interaction can be removed by ensuring that the interactions have been derived from no more than one host species and no more than one parasite species. Where the differential interaction is derived from quantitative data, identification of the genes involved is required to preclude the possibility of a Jeffrey DI being responsible. The possibility of an environmental DI is disproved either by demonstration of monogenic inheritance, or by evidence that the interactions found in the matrix were derived in one environment, at one time. The involvement of susceptibility genes is precluded by the

diagonal shape of the matrix, which provides evidence of matching resistance genes and virulence genes.

When environmental, polyphyletic, Jeffrey DI, susceptibility and immunity causes can be rejected, the transagonal 2 x 2 matrix provides a conclusive demonstration of a gene-for-gene relationship. It is the only 2 x 2 matrix which can do so. The transagonal 2 x 2 matrix is the smallest matrix which can be used to demonstrate a gene-for-gene relationship, and as such, a demonstration which relies on such a matrix has no degrees of freedom. This is why identification of the genes involved, or use of a larger matrix is advisable.

2.6 3 x 2 matrices.

A 3 x 2 interaction is obtained by the addition of one differential (either host or parasite) to a 2 x 2 matrix. To avoid repetition, only 3 pathotype x 2 pathodeme interactions are described; reversing the number of pathotypes x pathodemes would not alter the interpretation of a given matrix. Thus a 2 x 3 set of differential interactions can be analyzed in the same way as can a 3 x 2 set, and each will provide equivalent information.

There are three meaningful 3 x 2 sets of interactions

(Fig. 2.5). Others may be derived, but only with duplication of either rows or columns (redundancy). When two or more cultivars show the same resistance-susceptibility pattern, they must be treated as one pathodeme. Similarly, parasite isolates which exhibit identical virulence-avirulence reactions must be treated as one pathotype. This does not preclude subsequent discrimination when further interactions become available, just as "single" genes for resistance can sometimes be shown by further research to be composed of more than one heritable element (Crute, 1986).

Figures 2.5a and 2.5b can be used to demonstrate a gene-for-gene interaction. Both matrices contain a 2 x 2 transagonal, and so preclude the possibility of an environmental DI, and the possibility of abnormal host sensitivity to mycotoxins. The matrix shown in figure 2.5a also precludes the possibility of a polyphyletic, pathosystem, or immunity DI, (by showing that susceptibility to one pathotype exists in both pathodemes) and it is the smallest set of differential interactions which can be used to demonstrate a gene-for-gene relationship without genetic information, when the differential interactions used are derived from qualitative data. When the differential interactions used in the matrix are derived from quantitative data, demonstration of monogenic inheritance of resistance is required to preclude the possibility of a Jeffrey DI.

As with the quadratic check from which it is developed,

figure 2.5c does not disprove constant ranking, and consequently it cannot be used to demonstrate a gene-for-gene relationship.

Further extensions of this type of matrix (i.e. 4 x 2, 5 x 2 etc.) can be analyzed in terms of their constituent quadratic checks and 3 x 2 interactions. Such matrices are only as meaningful as the individual 2 x 2 and 3 x 2 matrices which can be found within them.

2.7 3 x 3 matrices

The next expansion is to a 3 x 3 matrix. Where the data used to derive the differential interactions used are qualitative, each of the 3 x 3 matrices shown in figure 2.6 is sufficient to demonstrate a gene-for-gene relationship, even in the absence of genetically derived evidence for the monogenic control of resistance and virulence. This is because no Jeffrey DI, or misinterpretation of horizontal resistance could cause this shape of matrix. For an immunity DI to be responsible, several species or formae speciales would have to be involved.

Figure 2.6a shows a matrix in which no pathodeme has more than one gene for resistance, and no pathotype has more than one gene for virulence. This matrix is an expansion of the transagonal 2 x 2 matrix, and it is proposed that matrices of

this type be identified by the number of interactions involved; thus this matrix becomes a 3 x 3 transagonal matrix. When the component differential interactions are derived from quantitative data, this matrix could result from a complex of Jeffrey DIs. To the knowledge of the author, no such complex has been reported. Nonetheless, if quantitative data are used, figure 2.6a must be supplemented with evidence of monogenic inheritance of resistance if a gene-for-gene relationship is to be conclusively demonstrated.

Figures 2.6b, 2.6c, and 2.6d show interactions in which pathodemes may have more than one gene for resistance, and pathotypes may have more than one gene for virulence. These matrices cannot be confused with alternative causes of differential interactions. The possibility of an environmental DI is removed by the complexity of the matrix, and the shape of the matrix precludes the possibility of abnormal sensitivity or horizontal resistance. The possibility of a polyphyletic or an immunity DI is also precluded by the shape of the matrices, since in each case the pathotypes are able to infect more than one pathodeme.

Other 3 x 3 matrices fall into two types; matrices which are analogous to the 3 x 3 matrices shown in figure 2.6 (but have rows or columns in different combinations), and those which do not demonstrate a gene-for-gene relationship. Those which do not demonstrate a gene-for-gene relationship do not

include a transagonal 2 x 2 matrix.

2.8 4 x 4 matrices

Two 4 x 4 matrices which can demonstrate a gene-for-gene relationship are shown in figure 2.7. Matrix 2.7a is an extension of the 3 x 3 transagonal matrix shown in figure 2.6a, and so it is called the 4 x 4 transagonal matrix. Strictly, this matrix could be caused by a series of Jeffry DIs in those cases where quantitative data has been used to derive the differential interactions in the matrix. To many readers, such a hypothesis may be less probable than that of a gene-for-gene relationship, and so the simplest hypothesis should have precedent. To remove all doubt, Mendelian inheritance of resistance or pathogenicity is necessary when such a matrix is based upon quantitative data. Such occurrences will be rare.

Matrix 2.7b is the repeating unit of the Person/Habgood matrix shown in figure 2.4. It is, by itself, a conclusive demonstration of a gene-for-gene relationship. All of the 3 x 3 matrices shown in figure 2.6, with the exception of 2.6a, can fit within the Person/Habgood matrix. Matrix 2.6a requires a change in nomenclature of the host and pathogen isolates before it can fit into the Person/Habgood matrix, but it can fit into the original Person (1959) matrix. Any 3 x 3

matrix taken from the Person/Habgood 4 x 4 matrix will provide a non-genetic demonstration of a gene-for-gene relationship. Any 4 x 4 matrix from the Person-Habgood matrix will demonstrate a gene-for-gene relationship, as long as that 4 x 4 matrix contains a transagonal matrix, and shows at least 2 other interactions which result in susceptibility.

2.9 Rules

As a result of the preceding discussion, five rules can be formulated.

1. Differential interactions used to demonstrate a gene-for-gene relationship must be derived from only one host species and only one parasite species. They should also be derived from data produced in a constant environment, or with an experimental design which takes environmental heterogeneity into account.

2. Demonstration of a gene-for-gene relationship requires a set of differential interactions which includes a transagonal matrix.

3. The 2 x 2 transagonal matrix is the smallest 2 x 2 matrix which can be used to demonstrate a gene-for-gene relationship. To do this, the matrix must be accompanied by evidence of monogenic inheritance of both resistance and virulence. Other 2 x 2 matrices do not conclusively

demonstrate a gene-for-gene relationship, even when accompanied by evidence of monogenic inheritance.

4. When evidence of monogenic inheritance is lacking, and the differential interactions are derived from qualitative data, the smallest matrix which can conclusively demonstrate a gene-for-gene relationship is a 3 x 2 matrix (figure 2.5a). Any of the 3 x 3 matrices shown in figure 2.6 (with the exception of 2.6a, when the differential interactions are quantitative) also demonstrate a gene-for-gene relationship without need of supporting evidence from genetic studies.

5. All matrices used to demonstrate a gene-for-gene relationship must fit within either the Person/Habgood matrix, or the Person DI matrix.

2.10 Conclusions

One of the advantages of using a set of differential interactions to demonstrate a gene-for-gene relationship is that the method can be applied to previously published host-parasite interaction data. This provides a quick means of demonstrating a gene-for-gene relationship, provided that suitable differential interaction data can be found.

Table 2.1 lists parasitic associations (and the source of the data) which provide enough interactions to construct a

Person/Habgood 4 x 4 matrix, thus demonstrating a gene-for-gene relationship in each parasitic association.

Table 2.2 lists associations (and the data source) which show a 3 x 3 matrix capable of demonstrating a gene-for-gene relationship. Gene-for-gene relationships have been suspected or implied to exist in many of these parasitic associations, but this is apparently the first time that demonstrations have been made. These tables are the result of an extensive, but far from exhaustive literature search and it is probable that data for additional demonstrations can be found. Much useful data may be unpublished.

Even when no pertinent data exist, the differential interaction analysis technique provides a means of demonstrating a gene-for-gene relationship which is more rapid and much easier than the traditional genetic proofs. Thus the differential interaction analysis technique has been chosen for the experimental investigations of this thesis.

Table 2.1 Host-parasite associations in which a gene-for-gene relationship can be demonstrated with a 4 x 4 matrix.

Parasite	Host	Parasite group	Source
Ascochyta pinodes	Pea	Fungus	Ali *et al*. 1978
Ascochyta pisi	Pea	"	Ali *et al*. 1978
Cercospora oryzae	Rice	"	Estrada *et al*. 1981
Phytophthora megasperma f. sp. *glycinea*	Soybean	"	Laviolette and Athow 1983
Phytophthora megasperma var. *oryzae*	Soybean	"	Haas and Buzzell 1976
Puccinia coronata	Oats	"	Bartos *et al*. 1969

Table 2.1 continued.

Therioaphis maculata	Alfalfa	Insect	Neilson and Don 1974
Globodera rostochiensis	Potato	Nematode	Cole and Howard 1966
Potato virus X	Potato	Virus	Cockerham 1955
Tobacco mosaic virus	Tomato	Virus	Pelham 1972

Table 2.2 Host-parasite associations which a gene-for-gene relationship can be demonstrated with a 3 x 3 matrix.

Parasite	Host	Parasite Group	Source
Bremia lactucae	Lettuce	Fungus	Channon and Higginson 1971
Colletotrichum lindemuthianum	Field beans	"	Tu *et al.* 1984
Synchytrium endobioticum	Potato	"	Ullrich 1958
Pseudomonas glycinea	Soybean	Bacteria	Fett and Sequira 1981
Xanthomonas campestris pv *oryzae*	Rice	"	Mew *et al.* 1982
Amphorophora rubi	Raspberry	Insect	Briggs 1965

Table 2.2 continued.

Nilaparvata lugens	Rice	"	Pathak and Saxena 1980
Heterodera avenae	Barley	"	Cook and Williams 1971

Figure 2.1 Constant ranking of disease reactions. 0 is the minimum amount of disease, and 4 is the maximum. The variation in disease reaction is quantitative.

		Hosts		
		A	B	C
Parasites	a	4	3	2
	b	3	2	1
	c	2	1	0

Figure 2.2 2 pathotype x 2 pathodeme matrices. Solid circles represent interactions which result in susceptibility, and hollow circles represent interactions which result in resistance. H = host, and P = parasite. The r and R notation represent possible host genes involved, and a, A, (avirulence) and v (virulence) represent possible parasite genes.

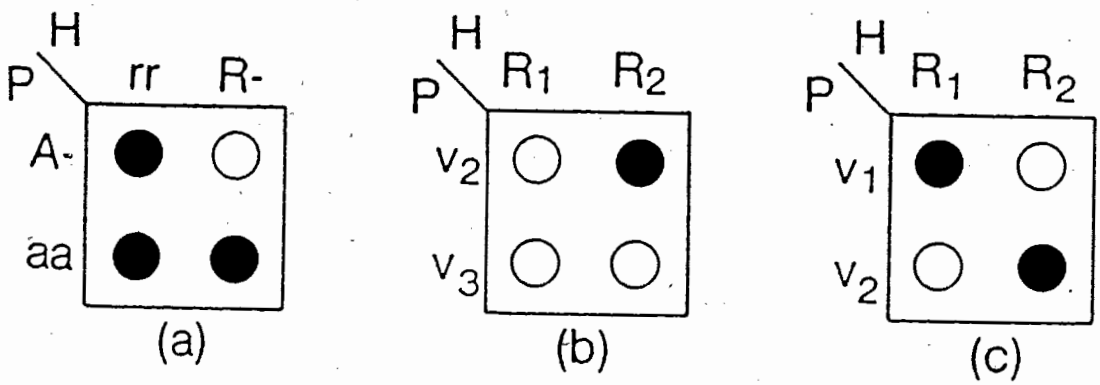


Figure 2.3 The Person differential interaction matrix. Solid circles represent interactions which result in susceptibility, and spaces represent interactions which result in resistance. From Person (1959).

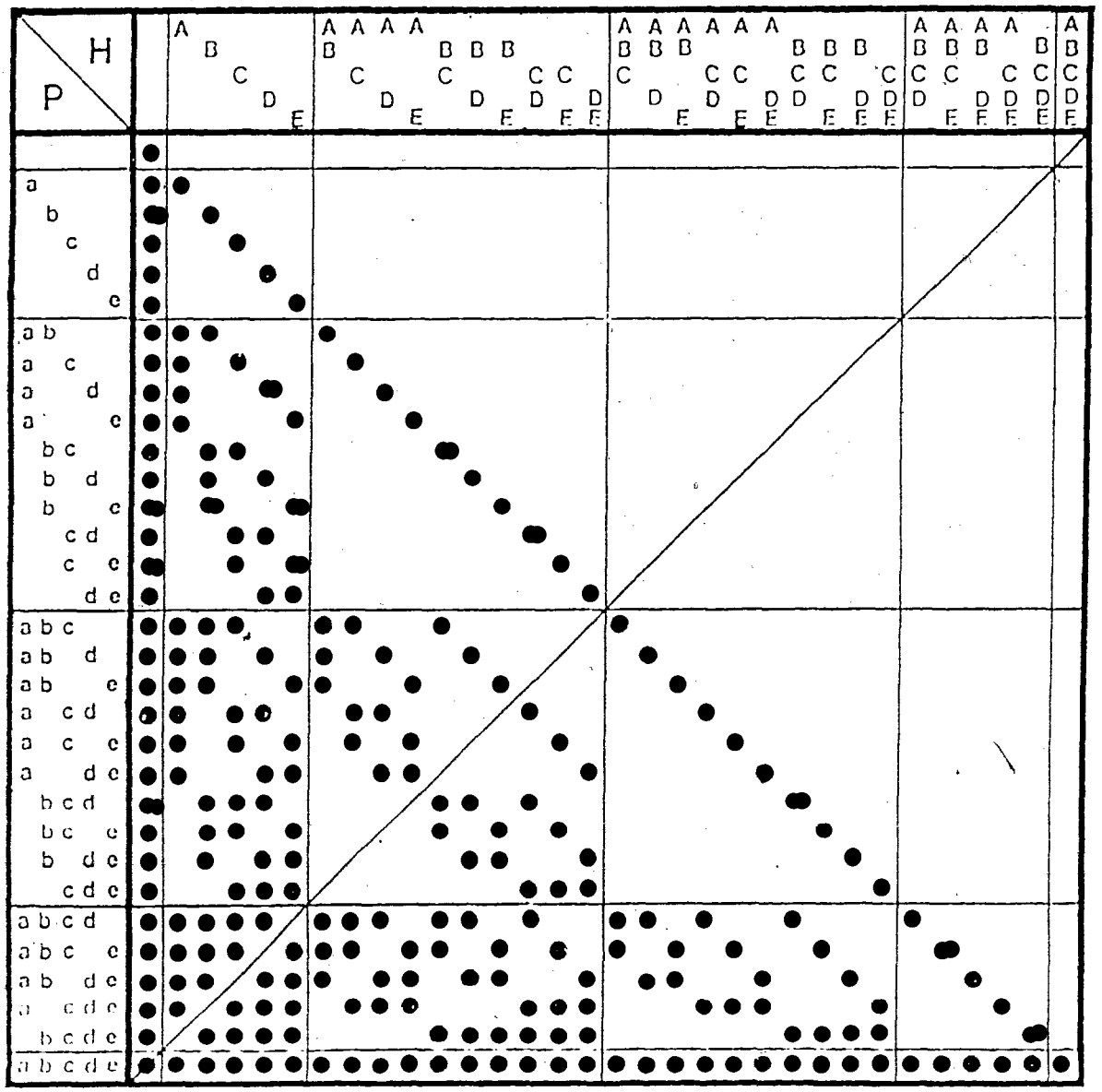


Figure 2.4 The Person/Habgood matrix. Solid circles represent interactions which result in susceptibility, and spaces represent interactions which result in resistance. The numbers are Habgood nomenclature. From Robinson (1976).

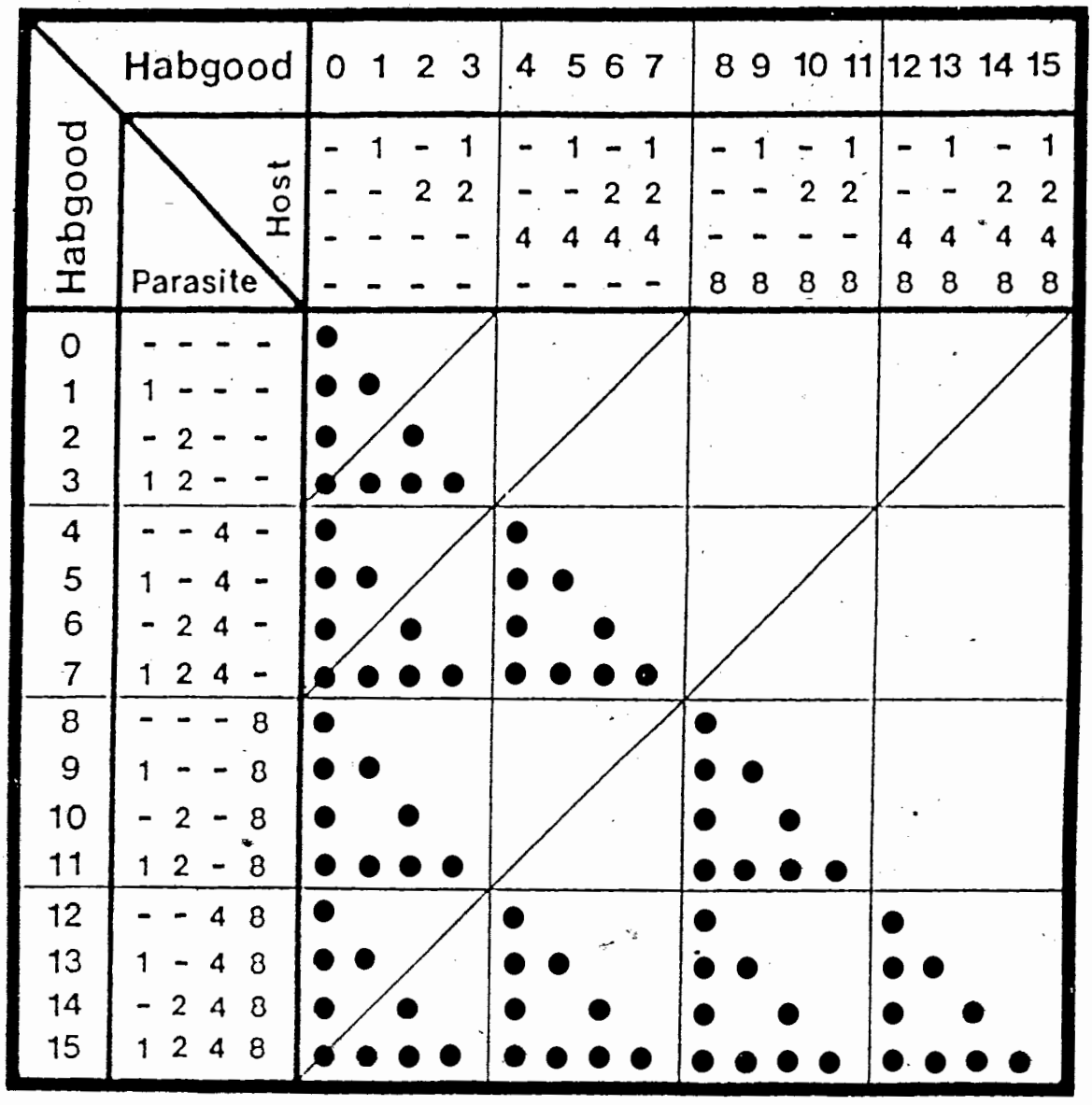


Figure 2.5 3 pathotype x 2 pathodeme matrices. Figures 'a' and 'b' show reversed disease reaction, and so demonstrate specificity. Figure 'c' shows no reversal of disease reaction, and so does not show specificity.

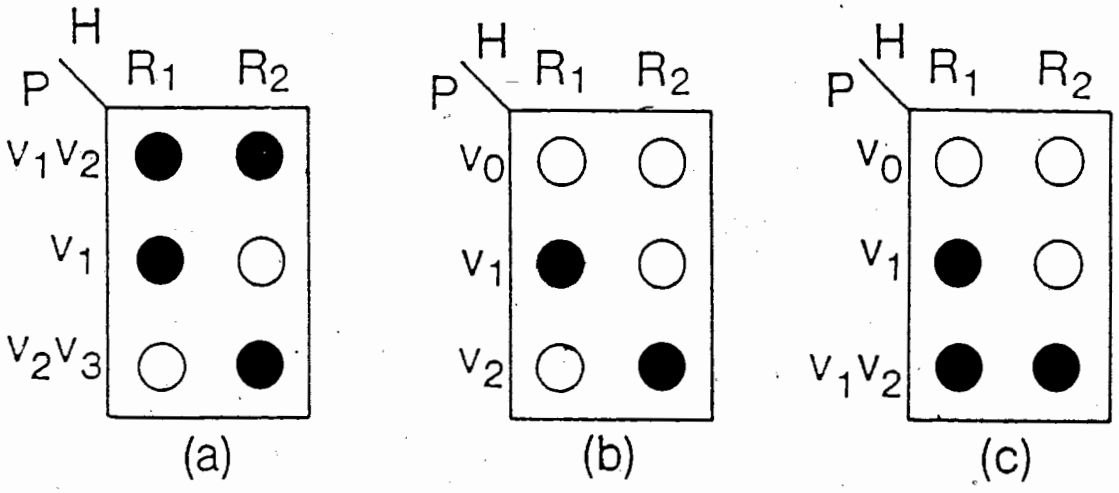


Figure 2.6 3 pathotype x 3 pathodeme matrices. All matrices show reversed disease reactions, and so show specificity. Matrix 'a' shows a situation where each pathodeme and each pathotype have only one gene for resistance (R), or one gene for virulence (v). The other matrices show situations where pathotypes and pathodemes may have more than one gene for resistance or virulence.

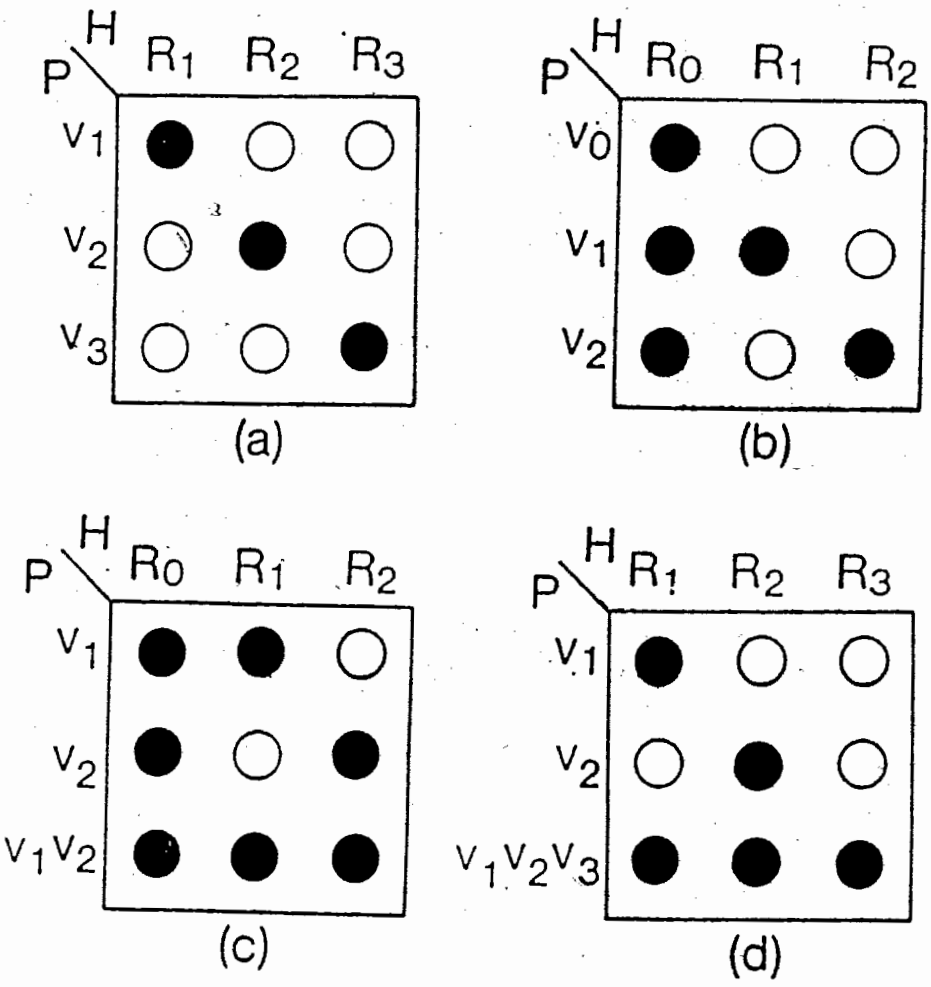
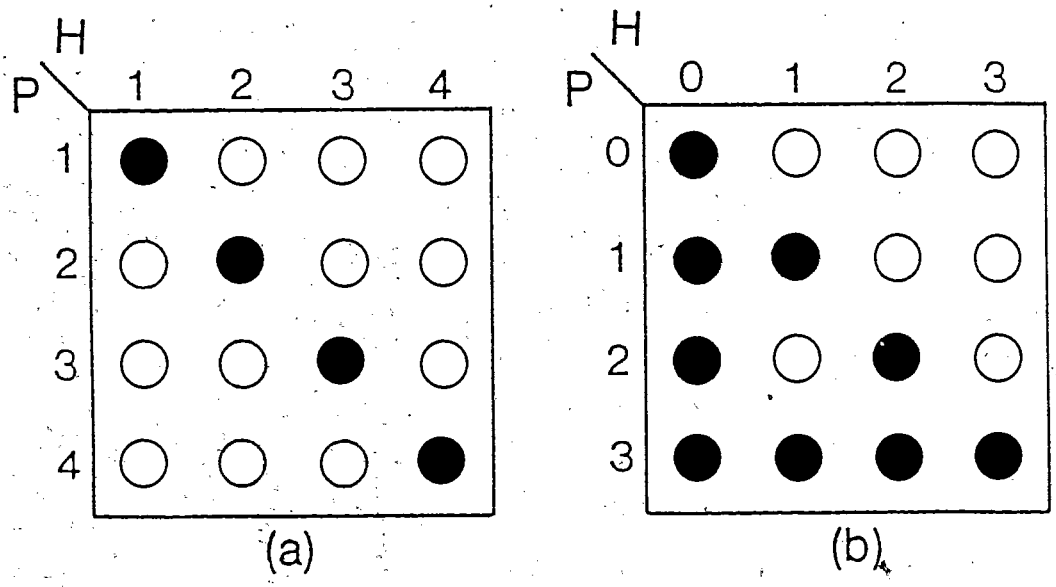


Figure 2.7 4 pathotype x 4 pathodeme matrices. Figure 'a' shows a situation where each pathodeme and pathotype posses one gene for resistance or for virulence. Figure 'b' shows the 4 x 4 unit from the Person/Habgood matrix.



Chapter 3

3.1 The *Ribes*-blister rust pathosystem.

Cronartium ribicola, the causal agent of white pine blister rust was introduced to western North America at Vancouver B.C. in 1910, in a shipment of diseased seedlings from France (Boyce 1961). Since then the disease has devastated white pine populations to the point that few white pines are now harvested in B.C., and plantings are more research oriented than commercial. The effect of *C. ribicola* on the *Ribes* populations has been less well documented, but appears to have been less destructive; one of the first blister rust control methods practiced was the "eradication" of the *Ribes* from white pine areas. Unlike the barberry eradication program, which now appears to have made an effective contribution to wheat rust control (Roelfs 1982), attempts at *Ribes* eradication have given only slight reduction of white pine-blister rust (Ostrofsky *et al*. 1987); the disease continues to be destructive on the pine host, and *Ribes* plants remain numerous.

Most research has been concerned with the pine hosts because these are the hosts of greater economic importance, but the *Ribes* hosts have not been ignored. All North American species of *Ribes* have been shown to be susceptible (Clinton and McCormick 1924). Mielke *et al*. (1937) showed variation among four species of

Ribes, and demonstrated variation in susceptibility within *Ribes petiolare*. Anderson and French (1955) observed variation in infection types on *Ribes hirtellum* and suggested the presence of races in *C. ribicola*. McDonald and Andrews (1982) showed that single aeciospore isolates varied in the fitness traits measured, but did not report any evidence of specificity. McDonald and Andrews (1981) used massed aeciospore (from single cankers on each of five trees) inoculations to show a significant aecium x *Ribes* clone interaction, and interpreted this as evidence of specific resistance in *Ribes hudsonianum* to *C. ribicola*. Unfortunately, their results showed high susceptibility or high resistance in the clones tested, but did not show the alternation of these phenotypes which is required to demonstrate specificity conclusively.

The following experiments were aimed at determining whether measurable differences in susceptibility and virulence occur in the *Ribes bracteosum* - *C. ribicola* pathosystem, and characterizing these differences wherever possible. *R. bracteosum* was chosen for its high susceptibility to *C. ribicola*, and its large leaf size, which enabled each leaf to provide many leaf discs. The primary interests were to determine which levels of the pathosystem, if any, show significant variation, whether host-pathogen specificity is present, and whether such specificity fits the gene-for-gene hypothesis.

3.2 Methods

The leaf disc technique used to investigate the Ribes-rust interaction is essentially a modified version of Clinton and McCormick's (1924) petri dish technique. All leaf discs were cut from detached R. bracteosum leaves, and measured 1 cm in diameter. Those leaves chosen to provide leaf discs were the most recent fully expanded leaf on a given stem, although experimentation showed that there appeared to be no differences in susceptibility or spore production among the four most recently opened leaves on a stem (Appendix 1). Most experiments involved one leaf/plant, and no more than two leaves/plant were used in any experiment. In all cases, discs were assigned to dishes in a random fashion.

Immediately after cutting, all leaf discs were placed on Whatman #1 filter paper which had been saturated with distilled water after being placed in a 9 cm diameter plastic disposable petri dish. Inoculation was by transfer of spores by fine gauge needle to a drop of distilled water placed on the surface of each disc.

After inoculation, all discs were transferred to a growth room, and placed in darkness for 48 hours. For the rest of the experiment a 16 hour photoperiod was maintained. The temperature was maintained at 16 - 21 C for the duration

of the experiment.

Each contained one representative leaf disc from each plant in the experiment, and each disc in that dish was inoculated with one spore source. Each inoculation consisted of approximately 250 spores, unless another inoculation dose is specified. An inoculation dose of 200-300 aeciospores has been shown to provide efficient and reliable infection (see Appendix 2).

Aeciospores were collected in the field (U. B. C. Research Forest, Maple Ridge, B.C.), from sporulating trees. Where aeciospores are specified as coming from a single blister, the aecial covering was not ruptured prior to aeciospore collection, and each collection was from a single blister, and unless otherwise stipulated, a single canker. Where aeciospore samples are described as being from a tree, they are composed of spores from many blisters and at least three cankers on a single tree. All spore samples were placed into glass vials which were then sealed and placed in a drying chamber. Aeciospores collected and stored at room temperature proved capable of germination for at least 6 weeks after the collection date, whereas the refrigeration technique of McDonald and Andrews (1980) was invariably associated with condensation and Penicillium contamination, rendering the spores inviable.

All uredospores were collected from infections on R. bracteosum discs. Thus all uredospores used were the product of the aeciospore sources.

All greenhouse Ribes were 2-3 years old, grown from

seed, and kept in the greenhouse. 'Wild' Ribes were well established plants located on Burnaby Mountain, Burnaby, B.C.

Data were recorded at two times the latent period, which is defined as the time interval (days) between inoculation and first sporulation (on any disc in the experiment) visible to the eye. Intervals of less than 2x the latent period were shown to miss some infections which showed up at the 2x latent period interval, while data collection at intervals greater than 2x the latent period showed no new infections on previously uninfected discs (Appendix 4). The interval of 2x the latent period was deemed to be the most efficient, as it maximized the number of infections recorded, and minimized the chance of reinfection of infected discs. The latter could exaggerate the size of the infection, thus biasing the results so as to increase the possibility of Type 1 error (incorrectly rejecting a true null hypothesis). The latent period was generally 14 days, plus or minus one day.

Data were recorded as presence and absence of infection, and as the number of mm2 covered by sporulation. The confidence level chosen for significance for all statistical tests was 95%.

Essentially, all tests were concerned primarily with determining sources of variability affecting infection of R. bracteosum discs, and the characterization of variability found. Presence/absence of infection data were judged to be the best form of data upon which to base conclusions about

qualitative vertical resistance. Data which recorded the amount of sporulation (number of mm2 covered by sporulation) were considered to be the best measure of horizontal resistance, or of quantitative vertical resistance.

3.3 Validation of the leaf disc bioassay.

The first experiments were aimed at determining the possible constraints on the leaf disc technique. These experiments determined that:

i. Ability to become infected and spore production did not vary within the first four most recently opened leaves on a stem (Appendix 1).

ii. Spore doses of 200-300 spores/disc provided consistent infection and spore production. (Appendix 2).

iii Leaf discs maintained on filter paper saturated with a nutrient solution (Hoagland's) showed similar infection to those maintained on distilled water, but produced significantly more spores (Appendix 3).

iv. Recording data at an interval of 2x the latent period enabled the maximum number of infections to be recorded, with minimum risk of bias due to secondary infection (Appendix 4).

Attempts to compare infection and spore production on leaf discs to that on intact plants failed to give results which would allow meaningful comparison, due to low inoculation efficiency on the intact plants. Low inoculation efficiency on intact plants is assumed to be due to difficulty in maintaining suitable environmental conditions for infection.

3.4 Variation between pine individuals as sources for *Ribes* infection.

The purpose of this experiment was to test the possibility that spores from different trees might vary in their ability to infect *R. bracteosum*, and that *R. bracteosum* individuals might vary in their ability to become infected by spores from different trees.

Leaf discs were selected from 18 greenhouse *R. bracteosum* plants, and aeciospores were collected from 6 pine trees. Each spore collection was made up of spores from ten or more blisters per tree. The leaf discs were arranged in a complete randomized block design, with six replicates (756 leaf discs, including controls).

This experiment showed a high level of infection (92.3%) on the inoculated discs (Table 3.1). Control discs showed no infection. Neither Freidman's test nor Logistic Regression analysis, using the presence/absence of infection results,

suggested any difference in the ability of R. bracteosum individuals to become infected (LR, $P \leq 0.15$), or any difference in the ability of spores from any pine individual to infect R. bracteosum (LR, $P \leq 0.55$). Nor was there any significant interaction (LR, $P \leq 0.91$).

The spore production data (# of mm2 of leaf disc surface covered with spores) were subjected to a 2-way analysis of variance, with interaction (Table 3.2). Significant differences were shown to exist between Ribes plants ($P \leq 0.001$) and between spore sources ($P \leq 0.001$). The interaction between the two factors was not significant ($P \leq 0.72$).

3.5 Variation between individual blisters from one pine tree as sources for Ribes infection.

The purpose of this experiment was to determine whether different blisters are equal in their ability to infect different R. bracteosum plants, and whether R. bracteosum plants are equal in their ability to become infected by spores from different blisters. Leaf discs were taken from 13 R. bracteosum plants, and inoculated with aeciospores from 4 blisters on one tree. The four blisters appeared to be the result of at least 2 stem infections. The experiment was given a complete randomized block design, with six replications (390 discs, including the control).

The infection results were subjected to Friedman's test and to Logistic Regression analysis (Table 3.3). With both tests the data show significant differences between blisters (LR, $P \leq 0.001$) and between Ribes plants (LR, $P \leq 0.001$). The interaction between the two factors was not statistically significant (LR, $P \leq 0.07$) at the 95% confidence level.

A $P \leq 0.07$ is not significant at the chosen 95% confidence level, but it strongly implies the existence of an interaction between Ribes plants and aeciospore sources. Thus the interaction cannot be said to be significant, nor can it be said that it does not exist. The best way to resolve this question is to repeat the experiment. This was not possible, due to insufficient inoculum from the original isolates. Instead, two additional experiments of a similar nature were performed.

The first repetition of the blisters x Ribes experiment involved aeciospores from 5 blisters (representing 5 cankers) and leaf discs from 6 R. bracteosum plants. The experimental design was a complete randomized block with 10 replications. Inoculations were as before. The infection data is shown in table 3.4.

The sporulation data were subjected to 2-way ANOVA, with interaction (Table 3.5). As before, the null hypothesis for the main effects must be rejected. The probability that the isolates are the same is calculated as being 0.02, and the probability that the Ribes samples are the same is even lower ($P \leq 0.001$). The null hypothesis for the interaction must

also be rejected ($P \leq 0.02$).

The second experiment tested aeciospores from 10 blisters on 6 R. bracteosum plants in a complete randomized block design with 5 replications. Inoculations were as before.

The Logistic Regression analysis of the presence/absence of infection data again indicated significant main effects ($P \leq 0.01$ for the blisters; $P \leq 0.001$ for the plants) (Table 3.6). The probability of an interaction occurring between blisters and plants was 0.07, the same as it was in the original blisters x R. bracteosum experiment.

The sporulation data were subjected to 2-way ANOVA, with replication (Table 3.7). The main effects were highly significant ($P \leq 0.001$ for both blisters and plants), as was the interaction ($P \leq 0.001$).

3.6 Variation between R. bracteosum individuals as sources of inoculum for R. bracteosum.

The experiments in this section were designed to test the null hypothesis that C. ribicola uredospores produced on different R. bracteosum individuals are equal in their ability to infect a range of R. bracteosum plants.

The first experiment used uredospores resulting from inoculation of Ribes with aeciospores mixed from several blisters on a single pine tree. Leaf discs from 10 R.

bracteosum plants were inoculated, in a randomized block design, with uredospores from 9 plants. Eight of the 9 plants which supplied uredospores were included in the test; these served as hosts for "self-inoculations. Each disc was inoculated with 25 - 50 uredospores.

The infection results were subjected to Logistic Regression analysis (Table 3.8). This test showed that although significant differences existed between the isolates ($P \leq 0.05$) and between the plants ($P \leq 0.005$), there was no statistical evidence for an interaction between the two factors ($P \leq 0.95$). However, confidence in these results is compromised by the overall low level of infection seen in the experiment as a whole (55.3%), and the low level of virulence of the "self-inoculations" in particular (57.5%).

Accordingly, another experiment of similar nature was conducted. Leaf discs from 15 R. bracteosum individuals were inoculated with 9 isolates of uredospores. Nine of the 15 Ribes plants were self-inoculation hosts. Each leaf disc was inoculated with 200 - 300 uredospores. The uredospores had their origins with 3 pine trees, and the experimental design was that of a complete randomized block with five replications (750 discs).

This experiment showed high levels of infection on the self-inoculations, indicating that inoculation had been effective (Table 3.9). Both Friedman's Test and Logistic Regression analysis of the infection results indicated highly

significant differences in infection between isolates (LR, $P \leq 0.001$) and between plants (LR, $P \leq 0.001$). Logistic Regression analysis also indicated a highly significant interaction between the two factors ($P \leq 0.001$).

The sporulation area data were subjected to ANOVA, two-way, with interaction (Table 3.10). The resulting analysis indicated that there were highly significant differences between R. bracteosum plants ($P \leq 0.001$) and between isolates ($P \leq 0.001$). There was also a highly significant interaction between the two factors ($P \leq 0.001$).

Would a random sample of wild R. bracteosum plants, without any known self-inoculations, show similar results? To test this, leaf discs from 15 wild plants were inoculated using uredospores produced on discs from 9 of the greenhouse plants infected in the previous experiment. The experimental design was that of a complete randomized block with 5 replications, as in the previous experiment.

Logistic Regression analysis of the presence/absence of infection results indicate the presence of highly significant differences between pathogen isolates ($P \leq 0.001$), and between plants ($P \leq 0.001$) (Table 3.11). The interaction between isolates and plants was also statistically significant ($P \leq 0.02$).

The amount of sporulation results were subjected to 2-way ANOVA, with interaction (Table 3.12). Highly significant differences were indicated to be present between isolates (P

$\leq$ 0.001) and between plants ($P \leq 0.001$), but there was no significant ($P \leq 0.55$) interaction.

3.7 Is the presence of a transagonal interaction indicated?

The purpose of the experiments described in this section was to test the hypothesis that statistically significant transagonal interactions could be identified in the R. bracteosum - C. ribicola pathosystem. Although subsets of the data from previous experiments can be selected to give a transagonal matrix, an increased number of replications of each interaction is required to determine the significance of any particular matrix. Finally, the interaction phenotypes producing the matrix must be repeatable.

The design of the experiments was similar to those preceding them, but the number of plants and isolates tested was decreased, so that the number of replications could be increased.

The first experiment involved inoculating leaf discs from 5 Ribes plants with uredospores from 7 sources, with 9 replications. The donor plants and the isolate sources were chosen on the basis of availability, not previous evidence of interaction. Three of the inoculations were self-inoculations.

The presence/absence of infection results (Table 3.13)

show one convincing transagonal matrix. The interactions used to make this transagonal matrix are highlighted with a *. The three self-inoculations (highlighted with a #) were successful enough to suggest good inoculation efficiency. The inability of any uredospore source to infect Ribes number 2 is of great interest, as these isolates proved virulent on other R. bracteosum. Unfortunately, further investigation of this plant was not possible, due to its inadvertent removal by university grounds maintenance personnel.

The second experiment in this section was designed to test the repeatability of the transagonal matrix demonstrated previously. Consequently, three of the isolates tested were those produced on leaf discs infected in the previous experiment, and three of the leaf disc donors were the same plants as tested in the previous experiment. Availability of suitable leaf tissue on the other host plants precluded an exact reproduction of the previous experiment. Thus one previously untested host individual and two previously untested isolates were added to those which had previously been tested, in the hope that a second transagonal matrix might be seen. The experimental design was once again randomized block, now with 12 replicates.

The results of this experiment are presented in Table 3.14. The reciprocal differential interaction involving Ribes 1 and 4, and isolates d and g in the previous experiment, was reproduced. The interactions which make up this transagonal

matrix are highlighted with a *. No other transagonal matrix is visible.

3.8 Discussion and Conclusions

Vanderplank (1984) has presented guidelines for the interpretation of biometric analyses of host-parasite systems. The host main effect (variation between hosts) should be interpreted in terms of horizontal resistance. Any variation in the parasite main effect (variation between isolates) should be interpreted as variation in aggressiveness. The interaction between host and parasite should be interpreted in terms of vertical resistance in the host, and virulence in the parasite.

These guidelines are used in this thesis, with the exception that the presence of significant interaction is not regarded as being interpretable only in these terms. Interaction between the host and pathogen is strongly suggestive of specificity, or specific interaction, but the cause may be other than vertical resistance. Alternative causes have already been outlined (Chapter 2).

The results obtained from the pine x Ribes experiment (section 3.4) indicate that significant differences in pathogenicity exist between populations of aeciospores produced on different trees, and in the susceptibility of individual R. bracteosum plants. This is indicated by the sporulation data, as

infection levels were high (92.3%) and effectively uniform. The absence of any qualitative interaction between isolates and Ribes plants indicates that if any specificity is present in this pathosystem, it is either absent in the samples tested, nonfunctional at this systems level, or is masked by the high infection rate of the overall experiment. Vanderplank (1968) has defined horizontal resistance as being that resistance which occurs in the absence of any specific interaction between host and pathogen. Thus the results of this experiment are interpreted as a demonstration of differences in horizontal resistance between R. bracteosum individuals, and differences in aggressiveness (sensu Vanderplank 1968) between isolates.

The apparent variation in aggressiveness between aeciospores produced on different white pine individuals is worth further consideration. There are several potential explanations for this variability, aside from variation in aggressiveness. One is that each tree is acting as a genetic sieve, only becoming infected by a subset of the pathogen population. Some trees may be infected by a larger subset than others, and so produce a wider range of spores virulent on Ribes bushes. Thus differing infection success/disc could produce an apparent variation in aggressiveness, though the genetic basis would be quite different. Should this be true, it could imply some linkage of pathogenicity on the pines with pathogenicity on the Ribes. Another explanation might be that trees differ in their ability to produce viable spores.

Host nutritional factors, host epiphytes, and hyperparasitism of aecial blisters are but a few of the possible causes of tree associated differences in aeciospore viability.

Variation in aggressiveness is the simplest hypothesis, and so this is the hypothesis favored by the author. Regardless of which hypothesis is most correct, such variation is potentially exploitable to reduce the C. ribicola population. This in turn could reduce disease.

Section 3.5 deals with the results of blister x Ribes inoculations. These inoculations provide consistent evidence of significant differences between blisters in their ability to infect and sporulate on R. bracteosum plants. The evidence of variability of infection within the Ribes plants sampled is also consistent. This is interpreted as being corroboration of the demonstration of horizontal resistance shown in the results of the previous section.

The interaction between blisters and Ribes plants is also a consistent feature of the experiments detailed in section 3.5, although the probabilities attached to this interaction vary from $P \leq 0.07$ to $P \leq 0.01$. This consistency forces the author to reject the null hypothesis of no interaction between the two variables.

According to Vanderplank (1984), a significant interaction is indicative of vertical resistance. As mentioned at the beginning of this section, such interaction can result from a situation which does not involve vertical

resistance; so while vertical resistance may be implied, it is not proven by the presence of an interaction. Certainly it is indicative of specific interactions within the pathosystem.

The presence of an interaction is of interest in another way, for it indicates that the blisters on a single tree are producing different pathogen genotypes. This is indirect evidence for the existence of heterothallism in C. ribicola (if individual cankers arise from infection by a single basidiospore). The presence of heterothallism has not been definitely demonstrated with this rust species.

The experiments detailed in section 3.6 add further corroboration to the existence of specific resistance in the parasitic association. Of particular interest, the results of this section demonstrate that uredospores produced on one plant may not be able to infect another, and that some host individuals are highly resistant to infection. Such evidence of highly discontinuous resistance strongly supports the hypothesis that vertical resistance is both present and operative in rust-Ribes association.

When such wide variability exists, and the cost of infection being partial or total defoliation for much of a season, it is reasonable to predict that natural selection for resistance would occur. Thus the Ribes population may not be as susceptible now as when the disease was introduced. This may mean that inoculum production for the pine populations

may also be decreasing, possibly reducing their levels of disease also.

The results presented in section 3.6, in which a repeatable reciprocal differential set of interaction phenotypes is shown, are perhaps the most important of this chapter. These results are conclusive evidence of a gene-for-gene relationship (as explained in chapter 2). The presence of a gene-for-gene relationship is the definition of vertical resistance. Thus, for the first time, vertical resistance has been demonstrated in a wild pathosystem.

Wild pathosystems have previously been suspected to involve gene-for-gene relationships, but the demonstration of such a relationship in a wild pathosystem proves that vertical resistance is not necessarily an artifact of domestication. Thus gene-for-gene relationships may be more common than previously expected.

The presence of vertical resistance in a wild pathosystem is of considerable importance to the managers of such pathosystems, and most of the world's forests involve wild pathosystems. The matching of vertical resistance (the so-called "breakdown" of resistance) in agricultural pathosystems has frequently been economically devastating. Agricultural pathosystems usually involve annual crops, and the investment of time associated with their production is small compared with that of forest crops. Thus a breakdown of vertical resistance in a forest pathosystem can be expected to be much

more costly than in an agricultural pathosystem.

The demonstration of a gene-for-gene relationship in the C. ribicola- R. bracteosum pathosystem is of further interest for two reasons. The first is that the pathogen is newly introduced, and has been in contact with the hosts tested for less than 80 years. Gene-for-gene relationships have previously been considered to require longer periods of coevolution, or the guiding force of artificial selection. The second reason is that this pathosystem is part of a heteroecious pathosystem, in which the other host may also be suspected of having vertical resistance. If so the C. ribicola- P. monticola pathosystem would be the first heteroecious pathogen shown to have gene-for-gene relationships with both hosts, and it would also be the first demonstration of a gene-for-gene relationship involving a gymnosperm host.

Table 3.1 The number of leaf discs, out of a maximum of six, which become infected following inoculation of leaf discs from 18 Ribes plants using aeciospores from six different trees.

	Aeciospore isolates					
Ribes plants.	A	B	C	D	E	F
1	6	4	6	5	6	6
2	6	6	6	5	6	6
3	6	6	6	5	6	6
4	5	6	6	6	6	6
5	6	6	6	4	6	6
6	5	4	6	4	6	5
7	4	6	6	6	6	6
8	6	4	4	5	5	5
9	6	5	6	6	6	6
10	3	4	6	4	5	3
11	5	6	6	4	6	5
12	6	6	6	6	6	6
13	6	6	6	3	6	6
14	6	4	5	6	6	5
15	6	5	6	6	6	5
16	6	6	6	6	6	6
17	6	6	6	6	6	6
18	6	5	6	6	6	5

Table 3.1, page 2. Logistic Regression results:

Source	deviance	d.f.	p
Isolates (trees)		5	0.15
Ribes plants		17	0.55
I x p		85	0.91

Table 3.2 Aeciospores from six different trees x 18 Ribes bracteosum individuals: summary of mm2 of leaf disc covered with uredospores.

	Isolates					
Ribes plants	1	2	3	4	5	6
1	20	17	28	25	37	32
2	25	37	33	33	36	41
3	17	13	25	17	24	22
4	28	20	39	46	47	48
5	19	24	40	15	43	34
6	13	14	23	8	16	21
7	18	30	39	38	46	38
8	18	16	18	22	20	19
9	14	18	43	30	32	35
10	7	25	19	14	26	17
11	13	19	33	25	34	30
12	16	28	36	31	31	33
13	27	31	28	19	42	41
14	19	18	22	25	25	27
15	32	34	48	39	53	33
16	20	23	33	24	35	29
17	19	18	32	38	29	29
18	22	27	41	46	44	35

Table 3.2, page 2. ANOVA results.

ANOVA table.

Source	DF	Sum-squares	MEAN-squares	F-ratio	Prob >F
Isolates	5	499.1	99.82	18.68	0.001
Plants	17	839.8	49.40	9.24	0.001
Isol x p	85	409.2	4.81	0.90	0.72
Error	540	2885.8	5.34		

Table 3.3 The number of leaf discs, out of a maximum of six, which became infected following inoculation of leaf discs from 13 *Ribes* plants, using aeciospores from four different blisters from one tree.

	Blisters			
Ribes	A	B	C	D
1	2	5	4	2
2	3	4	4	3
3	2	2	3	3
4	0	3	4	1
5	4	3	6	6
6	3	4	3	4
7	0	3	5	2
8	0	4	3	3
9	4	6	6	6
10	2	4	6	6
11	3	3	6	6
12	3	4	5	1
13	1	4	5	3

Table 3.3, page 2. Results of Logistic Regression analysis.

Source	d.f.	x2	P
Blister	3	34.2	0.001
Plant	12	45.0	0.001
Blister x plant	36	49.3	0.07

Table 3.4 The number of leaf discs, out of a maximum of 10, which became infected following inoculation of leaf discs from six *Ribes* plants, using aeciospores from five blisters on a single tree.

Ribes plants	Blisters				
	A	B	C	D	E
1	8	6	6	9	5
2	9	10	10	10	8
3	10	6	9	9	8
4	8	10	8	9	10
5	7	8	6	9	9
6	10	9	10	10	7

Table 3.5 Aeciospores from five blisters from a single tree x six Ribes bracteosum plants; summary of the area of sporulation (mm2) on each leaf disc.

	Blisters				
Ribes	A	B	C	D	E
1	25	6.25	17	16.75	13.5
2	44.5	71	52.5	72.5	46.5
3	50.75	8.75	23.25	44.5	42
4	69.25	92.5	61	90	59.25
5	17.5	10.5	9.5	7.5	17.75
6	44.25	25.5	23.25	51	25.75

Analysis of Variance results:

Source	DF	Sum-Squares	MEAN-Squares	F-ratio	Prob > F
Blisters	4	1.06	0.265	2.97	0.02
Plants	5	10.81	2.16	24.25	0.001
B x P	20	3.22	0.161	1.81	0.02
Error	270	24.06	0.089		

Table 3.6 The number of leaf discs, out of a maximum of five, which became infected after inoculation of leaf discs from six Ribes plants with aeciospores from 10 blisters from a single tree.

	Ribes bracteosum					
Blister	1	2	3	4	5	6
A	5	5	3	3	5	4
B	4	5	1	3	4	5
C	5	5	5	2	5	5
D	5	4	2	5	5	5
E	5	5	4	1	4	5
F	4	5	3	3	5	5
G	5	5	4	4	3	5
H	5	5	4	5	5	5
I	5	0	2	0	3	5
J	5	5	5	4	4	5

Logistic regression analysis results:

Blisters	8 DF	P = 0.01
Plants	5 DF	P = 0.001
Blisters x plants	40 DF	P = 0.07

Table 3.7 Aeciospores from 10 blisters from a single tree x six Ribes bracteosum plants: summary of the area (mm2) of the leaf discs covered by sporulation.

	Ribes bracteosum					
Blisters	1	2	3	4	5	6
A	26.75	21.0	9.75	1.5	16.25	26.25
B	23.25	37.25	6.25	2.7	7.5	30.5
C	47.25	35.0	21.75	1.0	25.5	25.5
D	46.25	28.25	8.25	3.0	14.0	18.5
E	29.75	23.5	9.75	0.2	6.25	17.25
F	31.5	43.25	23.5	1.2	28.5	33.5
G	47.5	35.75	11.0	4.25	16.0	27.75
H	61.5	36.5	18.25	2.7	20.25	31.25
I	32.75	0.0	4.0	0.0	9.5	39.25
J	54.5	57.0	19.75	2.5	10.25	39.25

ANOVA results:

Source	DF	Sum-Squares	MEAN-Squares	F-ratio	Prob>F
Blisters	9	4.001	0.445	7.54	0.001
Plants	5	19.98	9.996	67.79	0.001
B x P	45	5.08	0.113	1.92	0.001
Error	240	14.15	0.059		

Table 3.8 The number of leaf discs, out of a maximum of five, which became infected after inoculation with uredospores produced on nine different R. bracteosum plants. Numbers followed by "*" are the results of self-inoculations.

Isolates	Ribes bracteosum									
	1	2	3	4	5	6	7	8	9	10
A	5	3	3	4	4	0	3*	5	4	2
B	3	1	2	1	3	2	4	5	4	4*
C	2	3	2	4	4	3	4	4	2*	2
D	3*	2	4	3	1	3	4	3	4	3
E	0	1	4	2	3	1	3	5	4	0
F	4	3	3*	3	0	2	3	2	3	0
G	2	1*	3	3	1	4	3	3	4	0
H	3	1	3	3	0	3	4	5*	5	2
I	3	4	5	2*	2	3	4	3	3	1

Table 3.8, page 2. Regression Analysis results:

Isolates	8 DF	P = 0.33
Plants	9 DF	P = 0.001
Isolates x Plants	80 DF	P = 0.95

Table 3.9 The number of leaf discs, out of a maximum of five, which became infected after inoculation with uredospores produced on nine Ribes bracteosum plants. Numbers followed by a "★" are the results of self-inoculations.

Ribes	Isolates								
	A	B	C	D	E	F	G	H	I
1	4	1	0	4★	5	3	4	2	5
2	4	0	0	0	1	3	2	2	5
3	2	0	0	2	4	0	4	0	3
4	3	3	2	4	4	5	5	4★	5
5	4	2	0	5	4	5★	2	0	5
6	5★	1	2	5	5	4	4	5	2
7	0	0	0	0	0	0	1	0	0
8	4	2	4★	5	5	4	4	3	5
9	2	0	2	3	2	2	1	0	3
10	5	1	5	5	5★	4	4	1	5
11	5	0	2	5	3	5	4	2	5★
12	4	0	0	5	3	5	5★	3	5
13	2	2	2	3	3	5	5	1	4
14	1	1	1	3	3	0	1	1	4
15	4	4★	0	4	1	3	3	1	4

Table 3.9, page 2.

Logistic Regression results:

Isolate	8 DF	P = 0.001
Plant	13 DF	P = 0.001
Isolate x Plant	104 DF	P = 0.001

Table 3.10 Nine uredospore isolates x leaf discs from 15 Ribes bracteosum plants: summary of area (mm2) of leaf disc covered by sporulation.

Ribes	Isolates								
	A	B	C	D	E	F	G	H	I
1	30	5	0	9	41	10	25	7	24
2	4	0	0	0	3	3	2	2	5
3	2	0	0	4	5	0	5	0	5
4	8	7	5	20	11	18	28	10	22
5	15	2	0	20	13	22	8	0	8
6	29	4	9	20	25	18	21	23	10
7	0	0	0	0	0	0	2	0	0
8	28	6	9	18	13	10	8	15	32
9	4	0	4	3	3	3	2	0	4
10	19	4	18	23	24	16	9	0	18
11	5	0	3	5	8	5	5	5	7
12	9	0	0	12	12	12	28	6	25
13	6	3	3	7	5	8	10	1	12
14	1	3	4	9	6	0	3	3	11
15	15	4	0	6	5	9	19	5	8

Table 3.10, page two.

ANOVA results:

Source	DF	Sum-Squares	MEAN-Squares	F-ratio	Prob>F
Isolates	8	8.62	1.08	18.4	0.001
Plants	14	18.1	1.29	22.1	0.001
I x P	112	11.5	0.103	1.75	0.001
Error	540	31.63	0.059		

Table 3.11 The number of leaf discs, out of a maximum of five, which became infected after inoculation of leaf discs from 15 Ribes bracteosum plants with uredospores from nine Ribes plants, with no self-inoculations.

	Isolates								
Ribes	A	B	C	D	E	F	G	H	I
1	0	1	1	1	1	2	2	1	0
2	0	2	0	1	4	0	3	1	0
3	0	2	4	2	3	2	2	2	2
4	2	3	4	4	2	0	3	1	1
5	1	3	2	5	2	2	2	3	0
6	0	2	2	2	3	1	1	2	1
7	0	0	2	0	1	1	0	0	1
8	1	2	4	4	2	0	4	1	2
9	0	1	0	0	0	1	1	0	1
10	0	0	2	1	5	2	2	2	0
11	0	0	2	0	0	1	2	0	0
12	1	2	3	3	4	0	1	3	3
13	1	1	0	1	3	2	2	1	2
14	1	3	4	1	2	2	2	2	4
15	1	0	0	0	0	1	0	0	0

Table 3.11, page 2.

Logistic Regression results:

Isolates	8 DF	P = 0.001
Plants	14 DF	P = 0.001
Isolates x Plants	112 DF	P = 0.02

Table 3.12 Summary of the leaf disc area (mm2) covered with uredospores when inoculated with uredospores produced on nine Ribes bracteosum plants, with no self-inoculations.

Ribes	Isolates								
	A	B	C	D	E	F	G	H	I
1	0	4	20	5	4	5	2	4	0
2	0	4	28	0	33	7	0	11	0
3	26	16	12	19	33	5	22	16	0
4	11	3	20	0	22	24	19	19	12
5	0	43	14	23	24	24	6	20	2
6	2	12	7	4	8	2	0	5	0
7	1	0	0	3	5	0	2	1	0
8	6	9	22	0	19	21	26	14	4
9	3	0	3	2	2	2	0	3	0
10	0	21	14	5	11	4	4	1	0
11	0	0	2	1	0	0	2	0	0
12	10	6	3	0	17	3	18	7	3
13	3	5	7	6	8	0	0	2	1
14	23	20	17	12	26	11	15	22	5
15	0	0	0	1	1	3	1	2	2

Table 3.12, page 2.

ANOVA results:

Source	DF	Sum-Squares	MEAN-Squares	F-ratio	Prob>F
Isolates	8	3.82	0.477	4.2	0.001
Plants	14	9.81	0.701	6.16	0.001
I x P	112	12.46	0.111	0.98	0.55
Error	540	61.44	0.114		

Table 3.13 The number of leaf discs, out of a maximum of nine, which became infected when seven uredospore isolates were used to inoculate leaf discs from five Ribes plants. The numbers followed by a "*" are those which can be used to show a transagonal interaction. Those numbers followed by a "#" are the result of self-inoculations.

	Ribes				
Isolates	1	2	3	4	5
a	5	0	5	5	5
b	7	0	6	5	7
c	9	0	9	6	8
d	1*	0	9	7*#	8
e	1	0	5#	3	1
f	7	0	8	8	7
g	6*	0	5	0*	8#
h	0	0	3	0	5

Table 3.14 Repetition of the diagonal interaction between *Ribes bracteosum* individuals and isolates of *Cronartium ribicola*. *Ribes* 1, 4, and 5 are the same plants as represented by these numbers in Table 3.13. Isolates e, d, and g are the same isolates as given this designation in Table 3.13.

	Ribes				
Isolates	1	4	5	21	22
e	11	11	11	10	3
d	0*	10*	11	10	0
g	9*	0*	11	8	0
x	12	8	5	7	0

Chapter 4. Inoculation of detached white pine needles with *Cronartium ribicola* basidiospores.

4.1 Introduction:

White pine blister rust, caused by *Cronartium ribicola*, has devastated western white pine (*Pinus monticola* Dougl.) populations since its introduction to the west coast of North America in 1910 (Littlefield 1981). Thus it is ironic that infection of white pines under controlled conditions has proven to be difficult (Bingham 1972; Patton 1972; Kinloch and Dupper 1987). Infection requires that a basidiospore contact a host needle, germinate, enter a stomate, and infect the needle mesophyll (Clinton and McCormick 1919). The basidiospores are extremely sensitive to sub-optimal levels of temperature, light, and humidity (Littlefield 1981; Van Arsdel *et al*. 1956), and this sensitivity appears to be the cause of the difficulty in obtaining satisfactory infection under controlled conditions.

The inoculation technique used previously has involved suspending *Ribes* (the alternate host) leaves bearing telia over the pine plants to be inoculated. The *Ribes* leaves and the pine plants are then misted with tap water, and kept in darkness for 24 hours at 18 C (65 F)(Bingham 1972). This inoculation technique has several limitations (Bingham 1972; Patton 1972; Kinloch and Dupper 1987). First, these inoculations are usually performed in a greenhouse or

shadehouse because of the space required by the test plants. In such locations precise environmental conditions are difficult to maintain. Second, the technique often fails to provide successful infection, even when basidiospore germination takes place. When infection does occur, it is often non-uniform within an experiment, making comparisons difficult. Third, the technique requires large amounts of inoculum, which is a serious limitation for experiments requiring inoculum from specific isolates. Fourth, it is important to test mature trees as these are often suspected of possessing desirable levels of resistance. Tests on such trees can only be accommodated by first obtaining grafts (Patton 1961) or rooted cuttings (Williams 1987). There is need for an inoculation technique which is more reliable, more precise, and more convenient.

The development of the concentrated basidiospore suspension (CBS) technique provides a greatly improved means of delivering inoculum to the infection court. This method was first developed for inoculation of Pinus elliottii var. elliottii Engelm. and Pinus taeda L. with Cronartium quercum f. sp. fusiforme (Matthews et al. 1971; Matthews and Rowan 1972). Since then it has been adapted for use with Cronartium quercum f. sp. banksianae (Stewart et al. 1985), and Cronartium ribicola and Pinus monticola (Matthews 1984). The CBS technique provides a means of controlling inoculum density and of storing basidiospores for short periods of time. It also enables sources of inoculum derived from single

uredospores to be used more easily, because spores from a particular source can be used to inoculate a large number of hosts.

Leaf disc techniques, pioneered by Clinton and McCormick (1924), have proven very useful in investigating the response of many angiosperm plants to their pathogens. Experiments in which leaf discs serve as the host substrate involve cutting leaf discs from foliage and maintaining these discs on saturated filter paper in a petri dish. Leaf disc experiments have the advantages of being very space and time efficient, and permitting precise environmental control. An analogous method for conifers would involve the use of detached needles in place of leaf discs.

The author has determined that detached needles of western white pine maintained on filter paper saturated with distilled water in a petri dish at 21 C (70 F) and 12 hours light/day can remain viable for over a year, even when infected with C. ribicola. This longevity is more than sufficient to observe the results of successful infection with C. ribicola, as these become clearly visible in approximately 3-4 months.

The use of detached needles offers further advantages over the use of entire plants. Each host tree can be tested many times with only modest space requirements, and without having to clone the individual trees of interest. Many needles fit inside a petri dish, and many petri dishes fit inside a controlled environment chamber. This allows large numbers of

host genotypes to be tested under closely controlled conditions. Samples taken from trees of many different ages and environments can be easily accommodated.

4.2 Materials and methods:

The CBS technique of Matthews et al. (1971) and Matthews (1984) was used, with minor changes. Telia-bearing leaf discs (1 cm. dia.) were cut from Ribes bracteosum foliage, then fastened, telia down, to the inside of petri dish lids with a drop of dilute (0.1%) water agar. The bottom half of the petri dish was covered with 0.1% NaCl (laboratory grade) in double distilled water. The leaf discs were left suspended over the NaCl solution, in darkness, for 24 hours. The NaCl solution collects the basidiospores as they drop from the telia, and inhibits their germination. The basidiospores retain their viability after 24 hours in the NaCl solution, but longer exposures to the salt solution reduces viability (M. Morton, U.S.D.A. Forest Service, Priest River, Idaho, personal communication). Consequently, the collecting solution must be changed every 24 hours if viability is to be maintained. The telia-bearing leaf discs remain viable for many days, so that basidiospores may be collected many times from each source.

At the end of each 24 hour collection period the NaCl

solution was passed through a 3 um Millipore (TM) filter, and the residue on the filter was washed repeatedly with double distilled water at room temperature. Matthews (1984) recommends storage of basidiospores for several days at 0 - 1 C to stimulate germination. As refrigeration with the necessary control was not available (germination may be greatly reduced by temperatures below 0 C, and commences at 1 C), this step was omitted.

Washed basidiospores were immediately resuspended in double distilled water. A hemocytometer was used to estimate spore density, and the suspension was diluted to 3000 basidiospores/ml. This suspension was sprayed onto detached needles which had previously been placed on filter paper (Whatman's (TM) #3, 13 cm diameter) saturated with double distilled water, in 15 cm diameter plastic disposable petri dishes. Each petri dish received 2 ml of the spore suspension at each inoculation (68 spores/cm2). Control needles were sprayed with double distilled water only. After inoculation the petri dishes were placed on trays, and each tray was enclosed in a clear plastic bag for the duration of the experiment. The trays were placed in a controlled environment chamber maintained at 15 C (59 F) in darkness for 24 hours after each inoculation. Each dish was inoculated on 3 occasions, at 48 hour intervals (total inoculation dose = 204 spores/cm2). After the inoculation period the growth chamber was kept at 23 C (73 F) and a 12 hour photoperiod. The filter paper was resaturated with distilled

water at weekly intervals.

Two experiments were performed to test the CBS - detached needle technique. In the first experiment, basidiospores from six single uredospore-derived R. bracteosum infections were pooled to form one inoculum source. This was used to inoculate detached needles from six 18 month-old seedlings, and six 18 month-old rooted cuttings from 25 - 28 year old donor trees. Each dish contained one representative needle from each plant being tested, and each dish was replicated 10 times (240 needles used, including controls).

Whole plant inoculations were also performed using the same plants that supplied the needles for the experiment described above. The donor plants were kept in a greenhouse where they were misted with water prior to inoculation with the same spore suspensions as used to inoculate their detached needles. Needles selected for the control treatment were covered with thin plastic wrap to prevent inoculation with the basidiospore suspension. To prevent light-caused damage to the basidiospores these plants were kept in darkness (under black plastic) at 15 - 17 C (59 - 63 F) for 24 hours after each inoculation. After the inoculation period the plastic was removed, and temperatures varied between 11 and 23 C (52 - 73 F) during the course of the experiment. The purpose of this experiment was to serve as a comparison with the detached needle experiment described previously.

The second experiment used each of six single uredospore-derived Ribes lesions as separate sources of inoculum, and these isolates were used to inoculate detached needles taken from seven ten year-old trees. Each dish contained one replication, and each dish was replicated ten times (980 needles, including controls).

Red spots on the needles, frequently associated with successful infection of white pine needles (McDonald and Hoff 1975; Kinloch and Littlefield 1977) became visible three months after inoculation. The yellow chlorotic spots which are also associated with successful infection became visible one week later. At four months post inoculation no new spots became visible, but data from each experiment was recorded at six months post-inoculation to ensure that no late developing spots were missed. Six needle spots were dissected and examined under a light microscope to verify the presence of basidiomycete mycelium.

Because needles vary in length, the number of spots/needle is an inaccurate measure of infection. Consequently, data was recorded as the number of needle spots per unit length of needle, which was the least length of needle in each experiment (60 mm in the seedlings and rooted cuttings experiment, and 65 mm in the isolates x needles experiment). Data from the seedlings and rooted cuttings experiment was subjected to a log (x + 1) transformation (to better meet the assumption of normality of data), and a one-way analysis of variance (ANOVA). Data from the

isolates x needles experiment were subjected to a log (x + 1) transformation followed by a two-way ANOVA with interaction. The data concerning red infection spots was analyzed with Friedman's test (Zar 1974), as it could not be made to comply with the assumptions of ANOVA.

4.3 Results and discussion:

In the seedlings and rooted cuttings experiment, the greenhouse inoculations of intact plants were unsuccessful, showing only 7 infections on the 120 needles pre-chosen for data collection. Needle spots were much more numerous on the detached needles, and inoculated needles showed significantly more spots than did the control treatment (which showed no needle spots). The number of spots were almost equally distributed between the needles taken from the seedlings (47 spots) and the needles taken from the rooted cuttings (49 spots). Thus the null hypothesis of no difference in susceptibility between seedlings and rooted cuttings must be accepted. However, there were very significant differences ($P \leq 0.00$) between individual seedlings, and between individual rooted cuttings (Table 4.1). As the number of needle spots in this experiment is too small to allow separate analyses of yellow and red spots, all numbers in Table 4.1, and all stated results for this experiment, refer to yellow and red needle spots combined

(total number of successful infections).

Needles from physiologically older trees needles are generally thought to be less susceptible than younger tissue. Patton (1961) showed that grafts from mature trees were less susceptible to infection than were seedlings, and that susceptibility decreased with increasing age of the graft donor. The rooted cuttings used in this experiment were from mature trees (25 years old or older), and the cuttings have remained sufficiently old physiologically for two of them to produce male cones the following spring. It is possible that the small number of test plants and the high variation among plants obscures differences between seedlings and cuttings. Even if this is so, between tree differences appear to be much greater than age effects.

The isolates x trees experiment also showed adequate infection levels for statistical analysis (Tables 4.2 and 4.3). As with the cuttings versus seedlings experiment, each of the inoculated treatments showed significantly more needle spots than did the control treatment. The control treatment did show occasional needle spots, probably due to natural infection (from C. ribicola on wild Ribes plants) occurring in the shadehouse, prior to the needles being taken. The analysis (ANOVA) of successful infections (both red and yellow needle spots) reveals that there is a highly significant difference between trees ($P \leq 0.001$), no significant difference between isolates ($P \leq 0.15$), and a highly significant interaction between

isolates and trees ($P \leq 0.01$).

The highly significant interaction between isolates and pine trees can be interpreted as being indicative of specific susceptibility/resistance (Vanderplank 1984). The significant difference between trees (lowest #spots = 39, highest = 148) agrees with the findings of the seedlings and rooted cuttings experiment, and suggests that tree to tree variation in susceptibility is both large and frequent. The lack of a significant difference between isolates shows that the isolates tested are all able to infect a range of host individuals.

Specificity between C. ribicola and its sugar pine host has been demonstrated previously (Kinloch and Comstock 1981), and has been suggested to occur between C. ribicola and western white pine (McDonald et al. 1984).

McDonald and Hoff (1975) analyzed red and yellow needle spot frequencies in data from mass inoculations with unspecified, massed inoculum, and concluded that the yellow spots may be caused by a different race of rust than are the red spots. In this experiment, both types of needle spot appear to have been caused by basidiospores from single-uredospore isolates. If the different spot colors do reflect different races of the pathogen, those isolates which produce both types of spots must be heterozygous for this character.

Separate analysis of each colour of needle spot shows that their distribution differs significantly between hosts. Yellow spots, which are much more numerous than are red

spots, show significant differences between host individuals ($P \leq 0.001$) and a non-significant difference ($P \leq 0.17$) between isolates. The significance of these differences may be partly due to the significant interaction between host individuals and isolates ($P \leq 0.012$), but variation in the number of yellow spots/pine (lowest = 35, highest = 142) does appear to be real.

Red spots are also non-randomly distributed, with 42 spots of 88 being on 1 of the 7 hosts tested, and no red spots on one of the pine hosts. Friedman's test, using the red spot data, shows highly significant differences ($P \leq 0.001$) among host individuals, and no significant difference among isolates ($P \leq 0.93$). Thus there appears to be a strong host effect on needle spot colour, and little or no variation due to the pathogen isolates tested. The importance of this observation is not known.

4.4 Conclusions:

How accurately the detached needles reflect the reactions of needles which are still attached to the tree is not known, due to the failure to obtain significant levels of infection on the intact plants. At present one must either hypothesize that the differences shown by detached needles are representative of real differences, or that the needles from different trees react differently to being detached, and

that this strongly affects the susceptibility of the detached needles. The former is the simpler of the two hypotheses, and is the one used here. It is supported by the statistical significance of the results, and by the occurrence of typical infection symptoms.

The occurrence of infection symptoms typical of C. ribicola, and the absence of any visible fungal contamination in the inoculation suspension (examined while estimating basidiospore density with a hemocytometer), makes any involvement of other pathogens unlikely.

In summary, the CBS-detached needle technique appears to be capable of detecting significant variation within the C. ribicola - P. monticola pathosystem, and appears to provide more reliable infection than previously reported methods. The new technique appears to be able to do this while using lower inoculum densities and amounts than were previously reported. The results obtained using this technique indicate that great variation in susceptibility to C. ribicola exists in even small samples of western white pine populations, and that the technique may provide a means of rapid screening of trees suspected of having valuable resistance to needle infection. By providing reliable infection, the histology of infection should be easier to examine than it has been in the past. It may be that the technique will also be of use with other host species and other host pathosystems, as detached needles from Douglas fir (Pseudotsuga menziesii Dougl.) and

lodgepole pine (Pinus contorta L.) show similar longevity when kept in petri dishes.

The demonstration of specificity in the C. ribicola - P. monticola pathosystem has both theoretical and practical importance. The theoretical importance is that such specificity may suggest the presence of a gene-for-gene relationship, and a gene-for-gene relationship has yet been to be demonstrated with a gymnosperm host. The practical importance is that such specificity must be considered in any white pine improvement program.

The presence of a significant interaction between pine individuals and pathogen isolates can be interpreted as being indicative of the presence of vertical resistance (Vanderplank 1984). Experiments with sugar pine, in which resistance to C. ribicola was found to be determined by inheritance of a single gene (Kinloch and Comstock 1981), support this conclusion. Consequently it seems likely (though not certain) that gymnosperm hosts are capable of forming gene-for-gene relationships with at least some of their parasites. Certainly, the possibility of such a relationship must be given serious consideration by all managers of gymnosperm resources.

Table 4.1. Summary of the needle spots obtained by inoculating detached needles from six 18 month-old rooted cuttings and six 18 month-old seedlings of Pinus monticola with a suspension of Cronartium ribicola basidiospores from six single uredospore derived Ribes infections.

Rooted Cuttings						Seedlings					
1	2	3	4	5	6	1	2	3	4	5	6
9	4	7	10	14	5	11	2	11	3	19	1
Mean # spots = 8.2						= 7.8					
S. dev. = 3.7						= 7.1					

ANOVA results. Ho: all samples from different donors have the same number of needle spots.

Source	DF	Sum-Squares	Mean-Square	F-ratio	P
Among	11	1.55	0.14	4.78	0.001
Within	108	3.19	0.03		

Table 4.2. Summary of needle spots on detached needles from individual western white pines after inoculation with single spore derived isolates of Cronartium ribicola. Numbers outside parentheses represent the number of yellow needle spots. Numbers inside parentheses represent the number of red needle spots.

	Pines							
Treatments	1	2	3	4	5	6	7	Sum
Control	6	5	2(1)	4(4)	8	8	2	35(5)
Isolate #1	3(2)	6(6)	22	25(3)	16	24	17	113(11)
Isolate #2	3	6(6)	9	16(8)	19	15	17(5)	85(19)
Isolate #3	7(1)	5(1)	6	17(3)	20(4)	17	16(3)	88(12)
Isolate #4	10(1)	5(7)	5	20(13)	13	22	16	91(21)
Isolate #5	1	6(1)	7	14(4)	15(4)	25	18	86(9)
Isolate #6	5	4(1)	8	10(7)	23(3)	31	23	104(11)

Table 4.2, cont.

Sum yellow	35	37	59	106	114	142	109
Sum red	4	22	1	42	11	0	8
Sum r and y	39	59	60	148	125	142	117

Table 4.3 Summary analysis of variance results for individual western white pines x single uredospore derived isolates of *Cronartium ribicola* .

ANOVA results for red and yellow spots (summed), data given a log (x + 1) transformation. Control treatment excluded.

Source	DF	Sum-Squares	Mean-Squares	F-ratio	P>F
Isolates	5	0.30	0.0601	1.61	0.154
Pines	6	6.63	1.10	29.7	0.001
I. x P.	30	1.98	0.066	1.77	0.009
Error	378	14.07	0.037		

ANOVA results for yellow spots, data given a log (x + 1) transformation. Control treatment excluded.

Source	DF	Sum-Squares	Mean-Squares	F-ratio	P>F
Isolates	5	0.29	0.058	1.55	0.17
Pines	6	7.03	1.17	31.4	0.001
I. x P.	30	1.92	0.064	1.72	0.012
Error	378	14.11	0.037		

Results of Friedman's Test, using red spots only:

Isolates; t = 1.37 DF = 5 Prob (x > 1.37) = 0.93

Pines; t = 18.8 DF = 6 Prob (x > 18.8) = 0.001

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Appendix 1. Ability of the first four fully expanded R. bracteosum leaves to become infected with C. ribicola aeciospores.

Aeciospores were used to infect leaf discs taken from the first four fully expanded leaves of three Ribes plants. Ten leaf discs were taken from each leaf, and each leaf disc was inoculated with approximately 100 aeciospores. The inoculation results are presented in the table below, with leaf number 1 being the youngest leaf, and number four being the oldest leaf.

	Ribes			
Leaf	1	2	3	Sum
1	8	9	7	24
2	7	7	9	23
3	10	9	7	26
4	9	6	9	24

Although there did not appear to be any significant variation in the ability of aeciospores to infect the leaves tested, it was decided to standardize future experiments by using only the first fully expanded leaf. In those cases where the

first fully expanded leaf could not supply all the leaf discs required, the remaining leaf discs would be cut from the next oldest leaf.

Appendix 2. The number of C. ribicola spores required to provide efficient and reliable inoculation of R. bracteosum leaf discs.

Five leaf discs from each of six Ribes plants were inoculated with four spore densities; 5-10 spores, 25-50, 200-300, and 900-1000. All the aeciospores came from many blisters on a single pine. The number of discs showing infection at 2x the latent period (14 days) is shown below.

	Ribes					
Spore #	1	2	3	4	5	6
5-10	1	0	0	3	1	1
25-50	4	1	4	2	3	2
200-300	5	5	5	5	5	5
900-1000	5	5	5	5	5	5

The results show that the inoculation dose of 200-300 aeciospores delivered consistent and high (100%) infection of the

inoculated discs. The discs inoculated with fewer aeciospores were not so consistently infected, and the higher inoculation dose could give no increase in inoculation, and so was less efficient.

Appendix 3. *R. bracteosum* leaf discs maintained on filter paper saturated with distilled water versus leaf discs maintained on filter paper saturated with Hoagland's solution.

Leaf discs from five *Ribes* plants were each inoculated with 25-50 aeciospores from a single tree, with 24 replicates. Twelve replicates (60 leaf discs) were placed on filter paper which had been saturated with distilled water, and twelve replicates were placed on filter paper which had been saturated with Hoagland's solution. Table 1 provides a summary of the infection results (number of discs infected), and Table 2 provides a summary of the #mm2 sporulation data.

Table 1. Infection data.

	Leaf discs on Hoagland's					Leaf discs on distilled H20				
	Ribes									
Dish	1	2	3	4	5	1	2	3	4	5
1	2	2	3	2	3	2	3	3	1	2
2	2	1	3	0	3	1	2	3	1	2
3	2	3	0	2	1	2	3	3	2	1
4	3	3	1	2	3	2	3	3	2	3
sum = 41						sum = 43				

Table 2. Sporulation data.

	Leaf discs on Hoagland's					Leaf discs on distilled H20				
				Ribes						
Dish	1	2	3	4	5	1	2	3	4	5
1	8	11	43	2	35	5	11	14	1	8
2	3	2	25	0	15	2	7	13	1	8
3	12	20	0	4	13	3	11	22	3	9
4	10	13	9	4	15	4	9	17	2	25
sum = 244						sum = 175				

From these results it can be seen that while keeping R. bracteosum leaf discs on Hoagland's solution does not affect the number of disc's infected, it does appear to increase the amount of spores produced per infected disc.

Appendix 4. When to record the data.

For several experiments, data was recorded at several intervals. For the example used in this appendix, the latent period was 13 days, and the data was recorded at 1.7x the latent period, 2x the latent period, and 2.6 times the latent period. The tables below provide summaries of the infection results of nine Ribes plants which have been inoculated with uredospores from four different Ribes plants. Nothing has changed for each data set except the interval at which the data was recorded.

a. 1.7x latent period.

	Ribes								
Isolate	1	2	3	4	5	6	7	8	9
1	0	0	2	1	0	1	1	1	1
2	1	1	2	1	3	2	0	1	0
3	2	3	2	3	1	1	0	4	1
4	2	0	2	0	2	1	0	0	1

sum = 43

b. 2x latent period, and 2.6x latent period.

	Ribes								
Isolate	1	2	3	4	5	6	7	8	9
1	0	0	2	1	0	1	1	2	1
2	1	1	2	1	3	2	0	1	0
3	2	3	2	3	2	2	0	4	1
4	3	0	2	0	2	1	1	0	1

sum = 48

Recording data at the 2x latent period interval clearly identifies some infections which were missed at the earlier data collection, but at the 2.6x latent period interval no further leaf discs had become infected. Thus the 2x latent period was the shortest period at which data collection could detect all infected leaf discs.