VARIATION IN RESISTANCE AND VIRULENCE IN THE DISEASE INTERACTION BETWEEN MELAMPSORA RUST (*M. OCCIDENTALIS*) AND BLACK COTTONWOOD (*POPULUS TRICHOCARPA*)

By

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Variation in resistance and virulence in the disease interaction between Melampsora rust (M. occidentalis) and black cottonwood (Populus trichocarpa).

ABSTRACT

Disease severity, as expressed by spore production rate was compared in a test of fourteen clones of black cottonwood (Populus trichocarpa) by ten isolates of Melampsora rust (M. occidentalis), all collected from their natural pathosystem. Spore production rate was measured by average daily production on leaf disks during twice the latent period in days. The overall average uredospore production during the time from inoculation to twice the latent period was 650 spores/disk/day. Latent period ranged from 6 to 12 days with a median at 8 days. Clones as well as isolates differed significantly in their contributions to spore production rates, while there was no indication of specific differential interaction. The lack of qualitative resistance and virulence indicates that qualitative interactions do not play a major role in disease in this natural pathosystem. This finding holds the promise that black cottonwood resistance will not be devastatingly overcome when used in plantations.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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INTRODUCTION

As a rule, a parasite species has a very limited range of host species on which it can survive; but sometimes a parasite species is so specialized on a host species that only certain parasite races can parasitize a particular host variety. Knowledge of this specificity or physiological specialization may be very important to the plant breeder who seeks to produce varieties with resistance to disease, and to the plant pathologist who wishes to understand the source of disease stability.

In agricultural crops, one commonly observed phenomenon is that resistance incorporated into cultivars is overcome within a few seasons through physiological specialization by the pathogen. But, there are cases in which resistance has not been lost or even decreased over decades. In such cases, the loss of resistance through physiological specialization of the parasite apparently cannot or does not occur. Thus, the recognition of the type of resistance (Appendix A) in certain varieties stems from the history of their behaviour through periods of disease. With poplars and forest trees in general, such records are mostly non-existent. This sort of data may be most quickly gathered in the laboratory through artificial inoculations in order to assess the type of disease interaction involved.

The purpose of this study is to determine the type and extent of variation in resistance of Populus trichocarpa Torr. & Gray, and in virulence of Melampsora occidentalis Jacks, toward each other, when sampled from their natural pathosystem. Answers to two questions are sought: firstly, is there

physiological specialization (variation in virulence or resistance specific to particular host clones or pathogen isolates) such that rust isolates can be distinguished by specific differential reactions on a series of host clones, and vice versa? Secondly, does this degree of physiological specialization play a major role in this wild pathosystem?

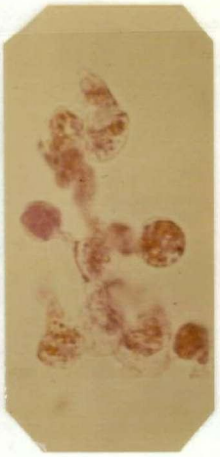
I selected this pathosystem for two reasons. Firstly, I wanted to study host:parasite interactions in a natural system where the host had not been under cultivation and put into plantations. Naturally growing black cottonwood (P. trichocarpa) was easily accessible and had the highly visible Melampsora leaf rust.

Secondly, I believe that black cottonwood will become an important commercial species in western North America in the near future. Poplars are excellent as short rotation intensive culture plantation trees (Hansen et al. 1983, McNabb et al. 1983), and black cottonwood is the largest of the North American poplars (Fowells 1965).

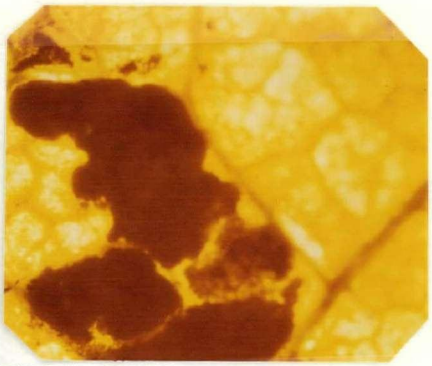
Black Cottonwood belongs to the poplar section Tacamahaca (FAO/IPC 1980) and is easily propagated both sexually and asexually (Muhle-Larsen 1970). Its natural range extends along the Pacific coast from Alaska to northern California and east to Montana. It is mostly found on bottom land, river bars, and forest meadows and streambanks, but it occurs throughout the plateau lands in north-central British Columbia (Fowells 1965).

Melampsora occidentalis (Melampsora rust) is a heteroecious eucyclic rust. Its life cycle (figure 1) is as follows: In spring, dikaryotic teliospores, which overwintered on dead

PYCNIAL DROPLETS exuded by monokaryotic mycelia from basidiospore infections contain pycniospores that are transferred between pycnia of different mating types in spring



BASIDIOSPORES (X 750) in spring infect conifer needles



TELIAL CRUSTS overwinter on fallen leaves. Teliospores germinate in spring to give basidia which germinate to give monokaryotic basidiospores

TELIA formed by dikaryotic mycelia in early fall

AECIA WITH LOOSE AECIOSPORES dikaryotic aeciospores formed from mycelia after pycnial transfer infect black cottonwood leaves in late spring

UREDIA of heavily infected leaf in late summer

UREDIA formed by dikaryotic mycelia from aeciospore infections or later from uredospore re-infections produce uredospores throughout summer

UREDOSPORE (X 200) BESIDE STOMATES will form germ tube to penetrate leaf through open stomate on abaxial surface

FIGURE 1: Life-cycle of M. occidentalis (read counter-clockwise)

leaves of black cottonwood, undergo karyogamy and meiosis, and then germinate to produce basidia. These basidia produce haploid basidiospores which can infect the needles of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) and other conifers to grow monokaryotic mycelia which form pycnia. After fertilization by pycnial transfer, dikaryotic aecia develop on the same needles, and aeciospores are produced. These dikaryotic aeciospores infect black cottonwood leaves beginning in late spring. The infections give rise to dikaryotic mycelia which produce uredia and uredospores to reinfect black cottonwood leaves. In late fall, the dikaryotic mycelia form dikaryotic telia which overwinter on the fallen black cottonwood leaves. This rust species can be manipulated sexually (pycnial transfer) and asexually (repeating uredospores). (Ziller 1974).

Host:Parasite Genetics

The terms used in this paper to describe host:parasite relationships are defined here. Resistance is the host component of disease interaction; it represents the ability of a host to hinder its parasite. Virulence is the parasite component of disease interaction; it denotes the ability of a parasite to reproduce on its host. Resistance, virulence, and the disease interaction may appear to vary qualitatively or quantitatively, depending on their genetic basis and the ease with which they may be observed (Scott et al. 1978 p.29). The genetic and epidemiological attributes of qualitative and quantitative interactions also vary greatly by the authority (Appendix A).

Qualitative interactions occur in systems where disease interactions fall into distinct non-overlapping classes. These are commonly termed compatible or incompatible, and may represent 100% or 0% disease classes respectively; or in cases where the disease interaction is not an all or nothing situation, the reaction can still be grouped into two classes, for example above 60% and below 40% disease. In any case, the different disease severities should be repeatably and significantly detectable. This is simply illustrated by a table:

		HOST CLONE A	B	C
	a	+	-	-
PATHOGEN ISOLATE	b	-	+	-
	c	-	-	+

+ compatible interaction (disease occurs)
- incompatible interaction (little or no disease present)
The differential interaction occurs here since the clones are differentially resistant and the isolates are differentially virulent.

The qualitative interaction forms the basis of recognition of physiological specialization in pathogen races and host varieties. Races are thus distinguished by differential reactions on host varieties as in the preceding table. Qualitative interactions have been conclusively demonstrated to arise from gene-for-gene interactions in a few pathosystems (Sidhu 1980).

According to the gene-for-gene relationship between a pathogen race and a host variety as postulated by Flor (1955), a race will be pathogenic to a variety if it possesses virulence genes which correspond to all the resistance genes in the variety. A race will be non-pathogenic or avirulent on a

variety if the variety possesses at least one resistance gene against which the race has no corresponding virulence gene. Thus a testing of various specified races against a host variety will give information on the existence of particular genes for resistance in the variety.

The other major interaction type is quantitative. Disease severity must be rated or quantitatively measured as it does not fall into distinct classes. The commonly ascribed features of this quantitative interaction are: polygenes, constant ranking, non-specificity, and stability in the interaction (Fleming & Person 1982). By constant ranking, a series of pathogenic races can be ranked for virulence, and this ranking remains consistent on any host variety; as well, host varieties can be ranked and will maintain the same ranking when tested against any pathogen race.

It is quite possible to find minor qualitative effects super-imposed over a basic quantitative system. This is well presented in a table by Scott et al. (1978 p.33):

VARIETY	ISOLATES	
	WYR 69/10	WYR 71/2
Hybrid 46	130	111
Joss Cambier	44	172

Quantitative interaction with reversed ranking. mg spores per 100 square cm leaf produced on seedlings of two wheat varieties by two isolates of Puccinia leaf rust.

However the qualitative differential interaction may not always be statistically significant, such as was found in the example above (Scott et al. 1978); and even when statistically significant, the differential interaction may have little

epidemiological impact, as host-specificity here gives only a slight advantage to the adapted race. The relative importance of qualitative vs. quantitative effects can be assessed by analyzing the components of variance.

It is easy to imagine either possibility, qualitative or quantitative occuring in this Melampsora-Populus pathosystem. If a qualitative system exists, such that cottonwood consists of a number of distinct host varieties which can be defined by their differential resistance to a series of pathogen races, then there would be in effect a natural multiline; each host tree or clonal group would be susceptible to some races but resistant to others.

Selection and testing for resistance could be difficult since certain clones might appear resistant simply because the test did not expose them to parasite races to which they were highly susceptible. If it were not known that qualitative interactions could occur in this pathosystem, and if these apparently resistant clones were grown in plantations, then virulent races would likely appear sooner or later and the qualitative resistance would be lost, possibly before the plantation reached maturity. (Kiyosawa 1982 p. 112).

On the other hand, the system might be quantitative with typical constant ranking. Experience with agricultural pathosystems has shown that such resistance, though incomplete, is quite stable and long-lasting. (Vanderplank 1982 p.77, Fry 1982 p. 200).

For this study, black cottonwood host clones will be tested against Melampsora pathogen isolates, and analysis of variance plus analysis of the components of variance should show whether the disease interactions differ significantly and whether there are specific differential interactions.

But no matter the outcome, it is not possible to conclude that there are no qualitative genes for resistance and virulence in this pathosystem. Possibly only a few trees carry specific powerful resistance genes and only a few pathogen races the corresponding virulence genes. However, if an analysis of variance shows no significant interaction, or if the components of variance show that the isolate-clone interaction term is small relative to the main effects, isolates and clones, then it may be concluded that such genes do not play a major role in this natural pathosystem. The null hypothesis can then be stated as follows: qualitative differential interactions play a major role in this natural M. occidentalis - P. trichocarpa pathosystem.

LITERATURE REVIEW

Many investigators have shown the variability of the Populus-Melampsora complex. The severity of Melampsora rust is a function of a complex of genetic (Heather & Sharma 1977), ontogenetic (Sharma, Heather & Winer 1980), and various environmental factors such as temperature and light intensity (Heather & Chandrashekar 1982). These Australian studies were

done with M. larici-populina on hybrid poplars. Shain (1976) with M. medusae on plantation P. deltoides in eastern North America, and Spiers (1978) with M. medusae and M. larici-populina on hybrid poplars in New Zealand reached similar conclusions. However, no extensive work has been done with the M. occidentalis - P. trichocarpa pathosystem.

Jokela (1966), Wilcox & Farmer (1967), Farmer & Wilcox (1968), and Farmer (1970) report that the heritability of rust resistance in the native Populus deltoides Marsh. population toward Melampsora medusae Thuem. is high. However Thielges & Adams (1975) rightly point out that heritability estimates are of no value where dominance between genes occurs. There are reports of both qualitative and quantitative resistance in the Populus-Melampsora complex.

Qualitative Resistance

Physiologic races in Melampsora larici-populina Klebahn. were first reported by van Vloten (1949). Then Muhle-Larsen (1963) reported that progeny resulting from many crosses of resistant and susceptible phenotypes of P. deltoides and P. nigra L. showed Mendelian segregation with respect to resistance toward Melampsora leaf rust.

Chandrashekar & Heather (1980) demonstrate racial specialization through the reaction of several clones of P. deltoides to mono-uredospore isolates of M. larici-populina . Several pathogen races could be recognized by qualitative distinct reactions on several clones of P. deltoides .

For Melampsora populnea (Pers.) Karst., Gremmen (1980)

reports that differential reactions occur for aeciospores from different aecial host species, so that there is at least species specificity if not race specificity.

Finally, a 1980 FAO/IPC report states that the results of crossing P. deltoides with P. nigra and P. trichocarpa a few generations removed from their wild ancestors indicate that resistance to Melampsora rust is a dominant character which depends on a relatively small number of genes (FAO/IPC 1980).

Quantitative Resistance

Thielges & Adams (1975) report much variation in resistance to Melampsora rust among and between families of open grown half-sib seedlings of P. deltoides collected from several eastern North American states. Pronounced differences in rust damage and rate of progression were related to geographic origin of the seed parent, thus there was a general constant ranking by the local wild rust population, which is indicative of quantitative resistance.

Eldridge et al. (1973) report that poplar cultivars in Australia differed in resistance with the greatest variation in disease severity between provenances and much less within. Again there is a general constant ranking of provenances.

Through testing of four M. larici-populina isolates against four Poplar cultivars, Heather, Sharma & Miller (1980) suggest that the resistance is polygenically based and caused by minor genes which interact with genes in the pathogen, although no genetic studies were done in this experiment.

METHODS

There have been no previous studies with this particular pathosystem to search for physiological specialization. Several such studies, however, have been done in other Melampsora-Populus pathosystems, but the differences between this pathosystem and other Melampsora-Populus pathosystems warranted several preliminary studies in methodology. These methodological studies: leaf age effects on susceptibilty, kinetin concentration effects on leaf disk senescence, and hyperparasite effects on uredospores, are presented in this methods section in the pertinent area.

Sample Size

Unlike many other Host-Pathogen studies, my specimens were to be drawn from wild populations and their disease interaction characteristics were not known. Thus a proper sample size was needed to ensure sufficient sampling:

$n = (t^2)\ (S^2)\ /\ E^2$

$S^2 = 1.2$ (in a disease rating system of 0 to 5, Farmer & Wilcox 1968. Similar value given in Jokela, 1966)

$E = 1$ (set to be significant difference in disease severity which I expect to be able to detect)

$t = 2.447$ (95% level of significance, 6 degrees of freedom)

$n = (2.447)^2\ (1.2)\ /\ (1) = 7$

This calculation was valid for quantitative systems, but as can be seen by the following calculations, the sample size held,

even conservatively, for qualitative systems. For qualitative systems, the sample size is chosen such that there is a 95% chance of selecting at least two distinct types (clones or isolates). If qualitative effects are important, the most common type should not comprise more than, for example, 50% of the population. The probability of selecting only one type in 'n' trees is .5 to the nth power. Hence, what is 'n' such that .5 to the nth is equal to 0.05 (i.e. 95% confidence)? The sample size 'n' is then found to be 5.

Therefore, at least seven trees and seven pathogen races were needed in sampling. As this was the minimum, it was decided that more than seven trees along with their Melampsora rust isolates were to be sampled.

Host selection and Telia collection

Eleven clones of black cottonwood were selected from around the Lower Fraser Valley in the autumn of 1982 and the spring of 1983. Telia of the autumn collected clones were also gathered at that time and stored outside in open-meshed bags. During the early part of 1983, two clones and their isolates were collected from the B.C. Interior and one from Calgary, Alberta. Listings of all clones and isolates as well as the collection and spore formation dates for all isolates are given in Appendix B.

The black cottonwood trees were chosen on the basis of being relatively isolated from other cottonwoods, and having attained reproductive age. Fallen twigs from the tree were usually collected, as reproductive buds and most of the branches

were in the higher portions of the tree. These abscised twigs due to cladoptosis are known to root readily in greenhouse conditions (Galloway & Worrall 1979). Leaves with telia were also collected from around the base of the tree to be used in virulence trials. Both collections required that the tree be isolated so that the identity of the twigs and leaves could be certain. Isolation was also required so that uredospore cycling should be on the same tree, presumably allowing build-up of a single Melampsora isolate. As isolation was the major criterion for sampling, trees with leaves that had no telia were also selected.

The requirement that the trees had attained reproductive age was added so that sexual crosses could be made if the parents were to exhibit qualitative resistance. Since black cottonwoods are mostly dioecious (Schreiner 1974), all crosses were not possible in any case. Following the method of Muhle-Larsen (1970) with twigs possessing reproductive buds set in flasks of distilled water, and with a paint brush to repeatedly dust pollen onto newly emerging female flowers, I did achieve one successsful cross which yielded 13 progeny. However, the parents did not differ significantly in overall resistance, so these progeny were not of interest.

Several trees from outside the Lower Fraser Valley were sampled to include geographic variation. Statistical analyses were later done with and without these clones and isolates. The first analysis was to show what type of variation in virulence and resistance may exist in the *P. trichocarpa* - *M. occidentalis* pathosystem at a species level. The second

analysis was to show what type of variation may occur in the system at a population level.

All collected leaves with telia were kept outside in open-meshed bags until April. Presumably, most of the telia on these leaves belonged to the pathogen race which was most successful on the tree during the past season. These telia were probably the result of auto-infection during the infection cycles within the tree. These telia could not be induced to germinate in the fall, probably because they probably require external overwintering stimuli to germinate in the spring (Longo et al. 1980, Von Weissenberg 1980).

The twigs were potted in sterilized soil and grown on long days in the greenhouse in order to produce enough leaves for the resistance trials. The daylength and average temperature in the greenhouse were 16 hrs and 20 degrees C. Greenhouse grown leaves have a low chance of wild infection, and none of my clones became infected in the greenhouse.

Uredospore Inoculum Preparation

In early April, leaves with telia were brought into the laboratory, washed in cold, running, tap water for one hour, and set on top of moist filter paper in petri dishes to incubate. Upon visible germination of the black telia giving a golden-yellow layer of basidia above the black telia, the leaves were suspended over pots of Douglas-fir seedlings planted in March and April. This procedure follows that of Ziller (1965). The assumption was made that all telia on the leaves of a single

cottonwood tree were closely related.

The inoculation of Douglas-fir seedlings by these basidiospores should yield pycnia on the upper surface of the Douglas-fir needles, but, unlike Ziller (1965), I did not find inoculation of Douglas-fir seedlings by basidiospores of M. occidentalis easily achieved. My inoculations yielded pycnia for two isolates, Imp and Pic (Appendix B). Possibly my telial isolates were adapted to an aecial host other than Douglas-fir and thus were not able to cause infection.

Inoculation was attempted with freshly washed telia that had not been set to germinate in petri dishes. These inoculations did not yield any pycnia. Attempts were also made to monitor sporefall by sporecounts of vaseline-coated coverslips set in pots of Douglas-fir for the duration of the 3-day inoculation; but contamination and the unevenness of the trapping media prevented accurate counts. The pots of Douglas-fir were kept in insect free cages to avoid spurious fertilization of pycnia.

Ziller (1965) has shown that M. occidentalis is heterothallic and thus it is possible to make controlled sexual crosses by pycnial transfers (Ziller 1959). Single transfers should lead to the formation of aeciospores, half of the time if there are only two mating types. The plan initially was to make only selfing crosses, and then, if the parental isolate lines proved to be of interest during later experimentation, non-selfing crosses would be made.

The transfer of droplets of pycnial fluid between pycnia derived from the same telial source yielded selfed progeny in

the form of aeciospores. This was successful for both isolates with pycnia, Imp and Pic.

From selfing crosses between pycnia of the same telial source, some spore lines were isolated. The aeciospores were inoculated onto their respective original host clone. A series of uredospore transfers followed to yield pure spore lines.

For all other isolates which could not be selfed due to lack of pycnia, uredia was collected in late summer from the original trees. (The collection dates are presented in Appendix B). The uredospores of several uredia of each leaf were spread onto green-house grown leaves of their respective original host clone. Several uredospore transfers followed before virulence trials.

I thought that these uredospores of late summer were likely those of the race which were well adapted towards their particular black cottonwood clones. If physiological specialization were occurring in this natural system, well adapted spores of late summer should be expected to exhibit it.

Virulence and Resistance Trials

The most recent fully expanded leaf of each clone was collected in the greenhouse. After surface sterilization with .35% sodium hypochlorite for 1 minute, each leaf was cut into ten 17 mm disks with a number 6 cork borer. Disks of one clone were placed into ten different petri dishes on top of number 3 Whatman filter paper saturated with a 5 ppm solution of kinetin. Each petri dish ended up with 14 disks representing the fourteen

clones (figure 2); In one experimental block, there were ten petri dishes, one for each isolate. The block was replicated at ten different times leading to ten randomized complete blocks as the experimental design. One block was discarded the eighth day after inoculation due to the unacceptably low infection. The inoculum quality for this block was suspect.

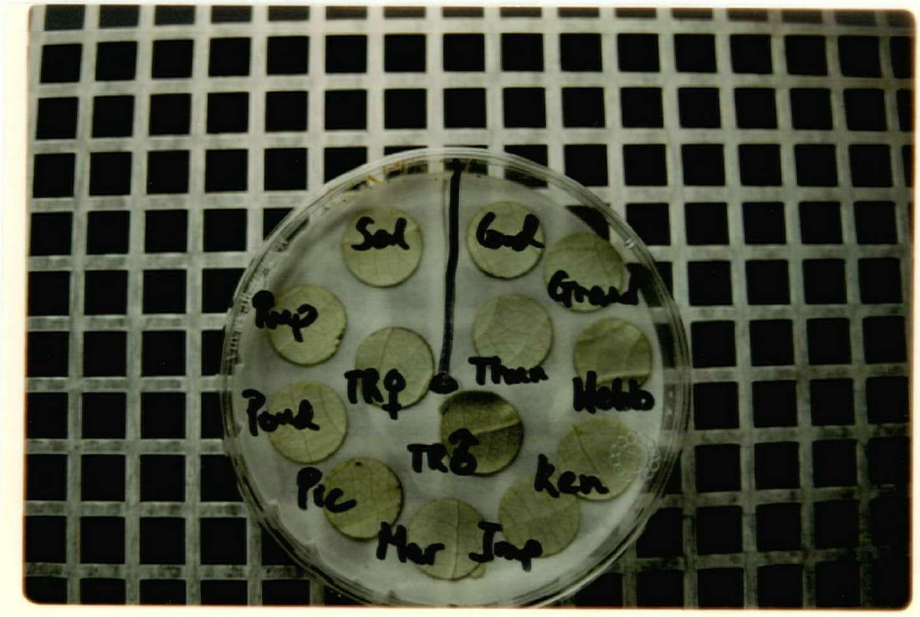


Figure 2: A single Melampsora rust isolate was applied to each of the fourteen clones of black cottonwood represented by 17 mm leaf disks in each petri dish.

To standardize the effects of leaf age and position on susceptibility to rust, the most recent fully expanded leaf was used. It has been suggested that a recently fully expanded leaf exhibits maximum susceptibility, and that subsequently susceptibility decreases with age (Sharma & Heather & Winer 1980, Lin & Edwards 1974). As well, the most recently expanded

leaf may be expected to senesce less quickly than older leaves.

A minor experiment was conducted to measure these age effects. Several cottonwood stems were stripped entirely of their leaves for use in this leaf age susceptibility study. Leaf halves were inoculated in petri dishes and incubated for 25 days at which time the disease severity was assessed by number of uredia and telia. The youngest leaves were the most resistant, and did not become diseased. The most recently fully expanded leaf was found to be the youngest or the second youngest leaf susceptible to Melampsora rust. (Appendix C).

The host clones were represented by leaf disks cut from leaves surface sterilized with .35% sodium hypochlorite for 1 min (Waller 1981). These disks were floated on filter paper in a 5 ppm kinetin solution in a petri dish, as has been done in other studies of the Melampsora-Populus complex (Shain & Cornelius 1979, Singh & Heather 1981)

In a small study, the effect of varying the concentration of kinetin was tested on halves of recently matured leaves. One half of these leaf halves were surface sterilized. The results showed that while there were no significant differences between sterilized and non-sterilized leaves, with the higher concentrations of kinetin (10, 50, or 100 ppm), heavy senescence occurred up to four times faster than with water. There were no great differences between water and up to 5 ppm kinetin. Chandrashekar (1982) found similar results.

Inoculations followed the method of Shain & Cornelius (1979). Uredospores were suspended in a .1% agar solution. Three droplets of suspension, each approximately .02 ml, were

inoculated with a micropipette onto each disk of one petri dish. Shain and Cornelius (1979) state that the optimum inoculation concentration is between 1250 to 5000 spores/disk (1.7 cm diameter). Indeed, I obtained heavy infection at 1300 spores/disk (1.7 cm diameter) in earlier inoculation tests.

Attempts were made to standardize inoculum concentrations to around 3000 spores/disk (three .02 ml drops of 50,000 spores/ml suspension) through a constant spore suspension absorbance reading (.05 absorbance units or 90% transmittance) on a spectrophotometer prior to inoculation; but the achieved range was 1000 to 20000 with an average at 6000 spores/disk. These spore counts were made on a haemocytometer after the inoculations rather than before, which may have contributed to to this great range in inoculum concentration.

The uredospore inoculum load was purposely set higher than the concentration at which heavy infection could occur on some clones. In inoculation curves (infection level vs. spore concentration), it is thought that slope decreases exponentially (concavely curvilinear) and reaches a concentration saturation level (slope goes to 0) where a greater concentration of inoculum does not cause greater infection. My objective was to ensure that inoculum concentrations onto all the clones exceeded their saturation point so infection level should be due purely to resistance and virulence rather than escape effects.

As a final check on proper inoculum load, a correlation was later made between inoculum concentration and single petri dish infection averages. (A single spore preparation was applied to a single petri dish). The results ($r = -.14$) showed no

significant correlation between spore concentration and infection levels, which meant that my inoculations were, as proper, in the range of saturation concentrations. As well, a graph of the two showed no apparent trends even among the lowest concentrations. (Appendix D).

After each petri dish inoculation, spores were plated onto 5% water agar to determine the percent germination. There was a imperfect fungus commonly associated with the uredospores which may have been inhibiting uredospore germination in the water agar plates. Readings of 0% germination were obtained, although infrequently, yet infection of leaf disks did occur from these same spore suspensions. It was suspected that a hyperparasite which was especially promoted in the water agar petri plate conditions was responsible. An unpublished experiment was done in the same lab to test the effects of this presumed hyperparasite.

This experiment was conducted by Elena Klein as a directed studies in the fall of 1983. She showed that although this presumed hyperparasite had a great effect in reducing infection when inoculated onto the leaf a week prior to uredospore inoculation, it had no effects in a 24 hr prior inoculation. However, there was considerable background variation in this experiment.

Hyperparasites and antagonists of Melampsora uredospores have been commonly reported (Bier 1965, McBride 1969, Omar & Heather 1979, Sharma & Heather 1981 & 1982), however their role in natural pathosystems is not known. These published studies were all conducted in laboratory conditions, and while

antagonists can be promoted in laboratory conditions, antagonistic concentrations may be uncommon in Nature.

Finally, the petri dishes of the main experiment were all placed into an incubation chamber at a temperature of 18 degrees C with constant cool white fluorescent light and watered every 3 days with distilled water. Krzan (1980) shows that optimal air temperature for germination of M. larici-populina uredospores is 16 degrees C, with higher temperatures more inhibiting than lower temperatures. Toole (1967) holds that the temperature optima for the germination of uredospores of M. medusae and M. larici-populina is 18 degrees C. Shain & Cornelius (1979) demonstrate that leaf disks of P. deltoides inoculated with M. medusae will have more severe infections at 18 degrees C than at 23 degrees C.

Rating System

Disease severity may be assessed by several parameters. In classical studies of host:pathogen relationships, infection type with defined categories of infection has been the main screening criterion, but many workers emphasize the importance of epidemiological characteristics such as number of pustules, incubation period, number of spores per pustule, and longevity of sporulation. (Zadoks & Schein 1978 p.7, Sharma & Heather 1979a). Number of spores would be an absolute measure of the virulence/resistance in the aegricorpus (host:pathogen association, Loegering 1978 p. 311); but, depending on spore production rates, the latent period (time from inoculation to

symptoms) could also have important epidemiological consequences. Thus a combination of the two, such as #spores produced / latent period, which is spore production rate, may give a better assessment.

Other studies in the Melampsora-Populus complex have used diverse measures for disease severity. In the multitude of publications by Heather and associates at the Australian National University, the measures used are: IPF(incubation period), ULD(uredia per disk), and USM(uredospores per mm^2). Measurements are made at 15 days after inoculation. Shain and Cornelius (1979) use total uredia and telia counts for disease severity, and measurements are made at 9 to 11 days after inoculation. Spiers (1978) similarly made uredial counts 10 days after inoculation.

In all of these studies, the particular reason for the date of measurement is not given. For each of my disks, which were observed daily, measurements were made at a period of twice the latent period so that all disease interactions could be measured at relatively the same stage of development. All uninfected disks were observed up to a minimum of 22 days.

The number of spores may be measured indirectly by measuring absorbance of a spore suspension with a spectrophotometer. (Both transmittance and absorbance scales are given on a Spectronic 20 spectrophotometer, but it is easier to read the linear transmittance scale because the absorbance scale is non-linear. After obtaining transmittance readings, one can easily convert them into absorbance units, since absorbance is the log of the inverse of transmittance). Sharma

& Heather (1979b) have shown that Melampsora uredospore concentration as measured by a haemocytometer has a high correlation (r=.998) with light absorbance at 640 nm of the same spore suspension. Spore suspensions for readings on a Spectronic 20 were made with modifications of Sharma & Heather's (1979b) procedure.

As latent period has a strong relationship with spore production, all disks were assessed upon reaching twice their latent period. Disease assessment took two forms: the first involved microscopic examination of the disks to count the number of uredia and telia. Uredia were rated into one of three diameter classes: <.2mm, .2mm to .5 mm, and >.5mm. The second method involved spore counts for disease severity. The disks were placed singly into test tubes with 1 ml of 5% Tween-20, and the tubes were vigorously agitated for 1 hour one hour on a Burrell wrist-action shaker. One and a half mls of water were then added to each tube to make the 2.5 mls needed for absorbance measurements on a spectrophotometer. The final 2% Tween-20 concentration did not detectably decrease transmittance in a Tween-20 absorbance test.

Random checks were carried out on one out of ten tubes with a haemocytometer to derive a correlation for absorbance (colorimeter) and spore count (haemocytometer). Spore suspensions for 154 disks were measured for absorbance and spore count. The derived equation was:

SPORE COUNT = 1228300 * ABSORBANCE - 7294,

which had a significant r=.91 (Appendix E). This negative y-intercept value indicates that there may have been discoloration

of the spore suspensions by the disks. Indeed, over 20 of these suspensions were made from disks with no infection, and these suspensions all showed a small degree of absorbance.

Since spore suspensions cannot take telia into account, a value of 500 spores was assigned to each telia to give the final equation:

SPORE COUNT = 1228300 * ABSORBANCE - 7294 + 500 * TELIA.

This value of 500 spores for each telium was decided upon after calculating that a medium size uredium released this number of spores in the disk shaking process.

Sharma & Heather (1979b) derived a similar equation of: UREDOSPORES = 1170000 * ABSORBANCE + 2800 for M. laricipopulina on hybrid poplars.

Analysis of variance was then performed on spore production rates as calculated from average spore production during twice the latent period. Analysis of variance was also carried out on total pustule count over twice latent period, on total spore count, and on latent period in days. Components of variance were then calculated for the variance sources used in the analysis of variance on average daily spore production to determine the variance contribution by each source.

RESULTS AND DISCUSSION

The spore production rates are presented in Table II. Latent period varied from 6 to 12 days with the median at 8 days. Out of 1260 disks, 235 did not become infected, although no single clone-isolate combination was without infection throughout the nine blocks.

Analysis of Variance

The analyses of variance for all specimens and for only Lower Fraser Valley specimens are presented in Table I. The first analysis was done to show what type of variation in virulence and resistance may exist in the *P. trichocarpa* - *M. occidentalis* pathosystem at a species level. The second analysis was to show what type of variation may occur in the system at a population level.

Both analyses showed that there were highly significant differences between clones of black cottonwood and between isolates of Melampsora rust, which meant that there were great differences in clonal resistance and in isolate virulence at both the population and species levels. The clonal means in spore production rate varied from 311 to 1008 spores/disk/day, and the isolate means varied from 283 to 1074 spores/disk/day (Table II). These analyses also showed that there were no significant interactions between clones and isolates. Thus there was no indication of differential interaction.

The significant F-value for blocks meant that significant variation was removed by blocking. This variation consisted of random effects, time effects (as blocks were replicated at

TABLE I: Analysis of variance for average daily spore production during twice the latent period, and isolate and clonal rankings (***).

a. For all specimens

SOURCE	D. F.	F-VALUE	F-PROB **
Block	8	6.942	.0000
Treatment	139	2.357	.0000
Isolates	9	5.318	.0000
Clones	13	14.442	.0000
Interaction	117	.929	.6884
Error	1112		
Total	1259		

Clonal ranking: * * *
Ken<Pond<Thun<Sal<Poco<Mar<Pic<Hebb<Rup<Imp<Gran<Cord<TrQ<Cal

Isolate ranking: * * *
IMP <GRAN <KEN <THUN <PIC <PRG <HEBB <ALK <RUP <POCO

b. For Lower Fraser Valley Specimens

SOURCE	D. F.	F-VALUE	F-PROB **
Block	8	5.003	.0000
Treatment	76	1.885	.0001
Isolates	6	8.600	.0000
Clones	10	5.319	.0000
Interaction	60	.870	.7457
Error	608		
Total	692		

Clonal ranking:
Ken<Thun<Sal<Pond<Mar<Poco<Hebb<Rup<Imp<Cord<Gran

Isolate ranking:
IMP <KEN <GRAN <THUN <HEBB <POCO <RUP

---

* Specimens from outside the Lower Fraser Valley
** F-prob is the probability that the null hypothesis, which states that there are no differences, is true.
*** The rankings result from Duncans multiple range tests (95% confidence), and all specimens underscored by the same line are not significantly different.

different times), variation between leaves of a single clone, and variation from separate preparations of the same isolate.

The lack of significant clone-isolate interaction coupled with significant differences beteen clones and between isolates means that there is constant ranking. From the isolate and clone rankings (Table I), it can be seen that although the absolute rankings differ slightly between the Lower Fraser Valley rankings and the all-specimens rankings, there are no statistically significant changes in ranking. Within one underlined group (Table I), specimens may change order between all-specimens and Fraser Valley specimens, but rankings do not change between groups.

Analyses of variance on other parameters of disease severity (average pustule production, total spore count, and latent period) showed the same results (Appendix F). Clones as well as isolates differed significantly, while there was no sign of differential interaction. These analyses were done because, depending on the resistance mechanism involved, it would have been possible for one parameter to demonstrate differential interaction while another didn't. For example, if the resistance mechanism were involved in impeding the penetration process, this could show up most clearly in latent period differences. Whereas if the resistance mechanism were involved in impeding parasitic growth and sporulation after penetration, this would probably show up better in total spore count than in latent period differences.

Physiological Specialization

Another presentation of the lack of detectable specialization or adaptation by the isolates toward their original host clones is given in Table II. It shows that in no case did an isolate produce more spores on its original host than on all other hosts. A t-test done on the difference in spore production rates between means of all original host-clone combinations and of all experimental combinations showed no significant difference between the two. Thus there was no indication of physiological specialization by pathogen isolates toward their original host clones.

TABLE II: Average daily spore production during twice the latent period for all isolates on all clones (units are spores/disk/day).

(clones) \ (isolates)	Alk	Gran	Hebb	Imp	Ken	Pic	Poco	PrG	Rup	Thun	MEAN
CAL	2152	639	2964	1020	1346	1740	3443	1270	2700	3245	2052
CORD	1333	813	1466	156	367	783	1362	903	1110	1669	997
GRAN	377	723*	348	392	1125	1034	1937	733	1767	1089	953
HEBB	1263	622	358*	316	631	623	672	885	817	470	666
IMP	955	1596	324	113*	547	560	583	671	1115	723	719
KEN	154	353	12	158	241*	361	591	490	405	341	311
MAR	890	376	953	221	237	433	870	1093	1143	311	653
PIC	645	261	804	509	473	1106*	813	773	602	481	655
POCO	678	406	868	89	688	847	644*	779	914	264	618
POND	339	178	181	196	364	456	642	313	456	203	333
RUP	770	316	490	398	328	868	1037	885	1091*	657	684
SAL	714	337	524	80	835	292	556	403	528	164	443
THUN	338	37	266	76	425	233	565	747	806	289*	371
TRQ	986	1322	1068	313	1516	987	1323	798	1159	606	1008
MEAN	828	570	765	283	652	737	1074	767	1044	751	747

*Original clone-isolate combinations

In my sampling of isolated trees, there was inherently a bias toward sampling those cottonwoods which had survived singly in their immediate area. They may have had some special characteristics such as resistance genes which allowed them to survive. In addition, isolates were taken from these same sampled trees, so that if physiological specialization were occuring, these isolates should surely demonstrate it. This can be contrasted to total random selection of both clones and isolates where the theoretical corresponding genes may not have been sampled. The sampling procedure thus further strengthens the result, that there are no signs of physiological specialization in this natural pathosystem.

Because I did not find differential interactions in the samples need not mean that there are none. Quite possibly differential interactions do occur to a minor extent in this pathosystem. Manipulation of the data by eliminating several clones and isolates lead to a significant F-value for isolate-clone interaction (Appendix G), but this data manipulation may be statistically unsound, and also this interaction may have no biological importance.

If differential interactions originating from physiological specialization do occur in this pathosystem, they are very rare. Mathematically, the sample size of 14 randomly selected clones allows me to say with 95% confidence that trees with qualitative resistance will play a part in 20% or less of all disease interactions in this pathosystem (calculations: what is x such that $(1-x)^{14} = .05$? x is found to be .20).

This is further demonstrated by an analysis of the components of variance: although this experiment contains considerable error, it was still possible to see the strongly significant differences between clones and between isolates (Table III).

TABLE III: Components of variance for the sources: isolates, clones, blocks, interaction, and error.

	percent variance accounted for: all specimens	Vancouver only
isolates	2.6%	5.7%
clones	11.5%	4.6%
blocks	3.5%	5.0%
isolate-clone interaction	0.0%	0.0%
error	82.4%	84.7%

If there had been no detectable differences between isolates or between clones, then one could claim that this experiment was not sensitive enough to detect differential clone-isolate interaction either. The relative variance contributions change somewhat for all sources except isolate-clone interaction, which remained at 0% (Table III). Calculations for components of variance are given in Appendix H.

Effects of Disease

Aside from the hypothesized genetics of disease interaction, this disease may have a profound effect on its host. In an unpublished experiment conducted in this lab by Susan Hruszowy on the effects of *M. occidentalis* on photosynthesis in black cottonwood, she found that

photosynthesis of diseased leaves was reduced significantly by 31% as compared to disease-free leaves on the same plant. This experiment was done in the greenhouse on leaves still attached to the plant.

Similar results have been reported in published studies. Uredopustules of some rusts act as sinks to accumulate metabolic products in competition with other plant parts (Wang 1961). Heavy infection by Melampsora rust causes premature senescence of leaves and defoliation (Schipper & Dawson 1974). Moderate rust infection can cause a 46% growth reduction (Widin & Schipper 1976). Defoliation has a great effect on stem height and basal stem diameter and consequently on the volume of wood produced by infected trees (Joly 1959, Widin & Schipper 1976). Donnelly (1974) reports that basal leaves of poplars export photosynthate mainly to the basal stem while higher leaves contribute more toward stem height; thus as basal leaves are most often first infected and abscised, basal stem diameter is greatly affected.

However, these figures for growth reduction are extreme and it may have been environmental interactions which allowed for such great severity. Palmberg (1977) found that poplar disease resistance to Melampsora rust was not significantly different between different clones on the same site, but was significantly different between sites. He also found very high genotype X environment interactions. Heather & Chandrashekar (1982) claim that environmental factors are most important in disease stability, and that light and temperature contribute more to variation than do cultivars or races.

Natural Pathosystems

There have been very few studies done on natural pathosystems. Robinson (1980) states that: "virtually no factual studies of a wild plant pathosystem have ever been undertaken" (p. 204), but does mention that two researchers have been working on a wild plant pathosystem in the Netherlands.

Other studies of wild plant pathosystems include Puccinia spp. on Avena spp. in Israel (Wahl et al. 1978, Dinoor 1977), two foliar diseases in a Trifolium repens population in Wales (Burdon 1980), Erysiphe graminis hordei on Hordeum sp. (Wahl et al. 1978), a leaf rust disease on Glycine sp. in Australia (Burdon & Marshall 1981), Puccinia spp. on Avena spp. in Australia (Burdon, Oates & Marshall 1983), and a rust disease of wild sunflower species (Zimmer & Rehder 1976). The problem with several of these studies is that the wild plants grew near their cultivated relatives, so that the selection pressure posed by the nearby uniform cultivars could have altered the pathogen population such that these wild pathosystems may not have been natural.

I could find no studies in the literature on natural forest tree pathosystems in this area of pathosystem theory. Even my study is not wholly natural since most of the specimens were selected from near urban settings. However, neither the host black cottonwood nor the pathogen Melampsora rust have been manipulated to any extent in the area, so that the unnatural and powerful selection forces arising from homogenous plantations have not had an effect.

Robinson (1979 p.21) speculates that qualitative resistance can evolve in natural discontinuous pathosystems but it need not do so. He also suggests that discontinuity (which refers to the continuity of the host:parasite interaction) would be the force which creates and maintains qualitative resistance, since continuous systems would have no use for qualitative resistance. However, unless the pathogen poses a great selection pressure on its host, qualitative resistance would not be necessary in the host in whichever system.

The M. occidentalis - P. trichocarpa pathosystem can be considered discontinuous, since the rust must cycle to the alternate host every year and there is normally no interaction between the rust and live cottonwood leaves during the winter. Foliage emerges each spring free of Melampsora rust. However, Melampsora rust does not appear to pose a continuous selection pressure on its host. Some years, nearly all trees are heavily infected; during other years, many of the same trees remain free from infection. Furthermore, even during years of heavy infection, major defoliation is not often observed and at any rate does not occur until late in the growing season.

Major resistance genes and virulence genes have been isolated from natural populations, and from this it is often inferred that major genes do have a role to play in disease systems that are stable. However another interpretation could be that these genes have a function other than in disease resistance.

It is possible to imagine that major virulence genes can be maintained in the pathogen population if there are powerful

selection pressures which act to favor distinct polymorphisms. One such pressure could be a physiological barrier such as lack of sexual reproduction. For example, in a microcylic rust without spermogonia, a mutation could give rise to a powerful virulence gene which allows its possessors to spread through the population. Lack of meiotic crossing-over would prevent gene combinations or a new genetic background for this major virulence gene where its effects would be modified, or where it would be incompatible.

Another selection presssure could be the powerful artificial selection posed by monocultures of a cultivar with a single set of strong resistance genes. Powerful virulence genes would then be required in the pathogen population for survival. Sidhu (1980 p. 396) lists 27 pathosystems in which gene-for-gene relationships have been implied, suggested, or demonstrated. None of these systems are naturally wild.

However the M. occidentalis - P. trichocarpa pathosystem which I studied is wild, and not under strong artificial selection pressures to produce major resistance or virulence genes. As well both partners are quite capable of outcrossing with other members of their species so no physiological pressure is present to maintain distinct disease interaction polymorphisms.

There is a valuable practical implication from these results. The lack of qualitative resistance and virulence in this system holds the promise that cottonwood resistance will not be devastatingly overcome when used in plantations. Cottonwoods reproduce very easily asexually by cladoptosis (twig

drop), and so it would not be uncommon to find neighboring individuals of the same genotype. Thus one could infer that even limited monoculture could be possible for plantations of black cottonwood. However, uniform plantations might strongly favor other diseases and disorders of black cottonwood.

There is also a theoretical implication of these results. Very little work has been done on natural pathosystems in the area of host:parasite genetics. The lack of qualitative resistance and virulence in the M. occidentalis - P. trichocarpa pathosystem indicates that qualitative interactions do not play a major role in disease in this system. However, many more studies of other natural pathosystems are required before making the general conclusion that qualitative interactions arising from physiological specialization due to gene-for-gene effects do not occur in natural pathosystems.

CONCLUSION

An analysis of variance of average daily spore production by 10 isolates of *Melampsora occidentalis* on 14 clones of *Populus trichocarpa* showed no indications of physiological specialization. Clonal resistance as well as isolate virulence differed significantly. Analysis of variance of total spores, average daily pustule production, and latent period all gave this same result. The overall average spore production during the time from inoculation to twice the latent period was 650 spores/disk/day. Latent period ranged from 6 to 12 days with a median at 8 days.

The sampling technique was biased toward selecting specimens which had the opportunity to be physiologically specialized, since isolates were collected along with their hosts in late summer; yet this specialization was not found. The lack of qualitative resistance and virulence indicates that qualitative interactions do not play a major role in disease in this system. This finding holds the promise that cottonwood resistance will be of a durable nature and not devastatingly overcome when used in plantations.

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APPENDIX A: Various labels have been applied to the resistance, virulence and disease interaction. The following list is adapted from Browning, Simons & Torres (1977):

VERTICAL	HORIZONTAL	Vanderplank 1963
PERPENDICULAR	LATERAL	Vanderplank 1963
SPECIFIC	NON-SPECIFIC	Zadoks 1961
MAJOR GENE	MINOR GENE	
MONOGENIC	POLYGENIC	
OLIGOGENIC	MULTIGENIC	
MULTIPLE ALLELE	MULTIPLE GENE	
QUALITATIVE	QUANTITATIVE	
HIGH	LOW,MODERATE	
SEEDLING	ADULT	
HYPERSENSITIVE	NON-HYPERSENSITIVE	
PROTOPLASMIC	NON-PROTOPLASMIC	
DISCRIMINATORY	DILATORY	Browning et al. 1977
DIFFERENTIAL	UNIFORM	Vanderplank 1963
	FIELD	
	DURABLE	Johnson 1979
	TOLERANCE	

These are but some of the terms which have been used to describe resistance, virulence, and disease interaction. Within each column the terms given are at least partially synonymous. Publications in the field of host-parasite genetics most commonly use the terms vertical and horizontal resistance. However, these terms have become burdened with both genetic and epidemiological definitions which, according to many people, are not necessarily equivalent (Johnson 1979, Ellingboe 1981).

Furthermore, the two columns represent two distinct categories to some authors (as above) while others do not see a distinction and view the corresponding terms as representing two extremes of a continuum (Nelson, 1978; Ellingboe, 1975). The terms used in this paper, qualitative and quantitative, are not necessarily synonymous with other terms in the same column, and should be read solely with the definitions given them in this paper.

APPENDIX B:

1. LISTING OF ALL CLONES AND ISOLATES

Name	Isolate	Clone	Location
ALK	X		Allison Lake, B.C.
CAL		X	Calgary, Alberta
CORD		X	Corduroy Trail, U.B.C Endowment Lands
GRAN	X	X	Grandview & Nanaimo Str, Vancouver
HEBB	X	X	Hebb Str. (Renfrew & 10th), Vancouver
IMP	X	X	Imperial Drive & 16th, Vancouver
KEN	X	X	Boundary & Nelson Str, Vancouver
MAR		X	Fraser Viewpoint, U.B.C.
PIC	X	X	Picnic Point, Kamloops, B.C.
POCO	X	X	Port Coquitlam, B.C.
POND		X	Ponderosa Cafeteria, U.B.C.
PRG	X		Prince George, B.C.
RUP	X	X	Rupert & Grandview, Vancouver
SAL		X	Salish Trail near B.C. Res., U.B.C.
THUN	X	X	Thunderbird Stadium, U.B.C.
TRQ		X	Tranquille Farm, Kamloops, B.C.

These clones did not survive in the greenhouse: ALK and PRG.
These isolates were not present: Cal,Cord,Mar,Pond,Sal,TrQ.

2. ISOLATE COLLECTION AND FORMATION DATES

	COLLECTION		FORMATION			
Isolate	Telia	Uredia	Basidia	Pycnia	Aecia	Uredia
Alk		10/83				
Gran	10/82	8/83	7/83			
Hebb		8/83				
Imp	10/82		20/5/83	31/5/83	16/6/83	3/7/83
Ken	11/82	8/83	6/83			
Pic	10/82		18/6/83	27/6/83	15/7/83	27/7/83
Poco		8/83				
PrG		8/83				
Rup		8/83				
Thun	10/82	8/83	7/83			

APPENDIX C: Leaf age susceptibility study

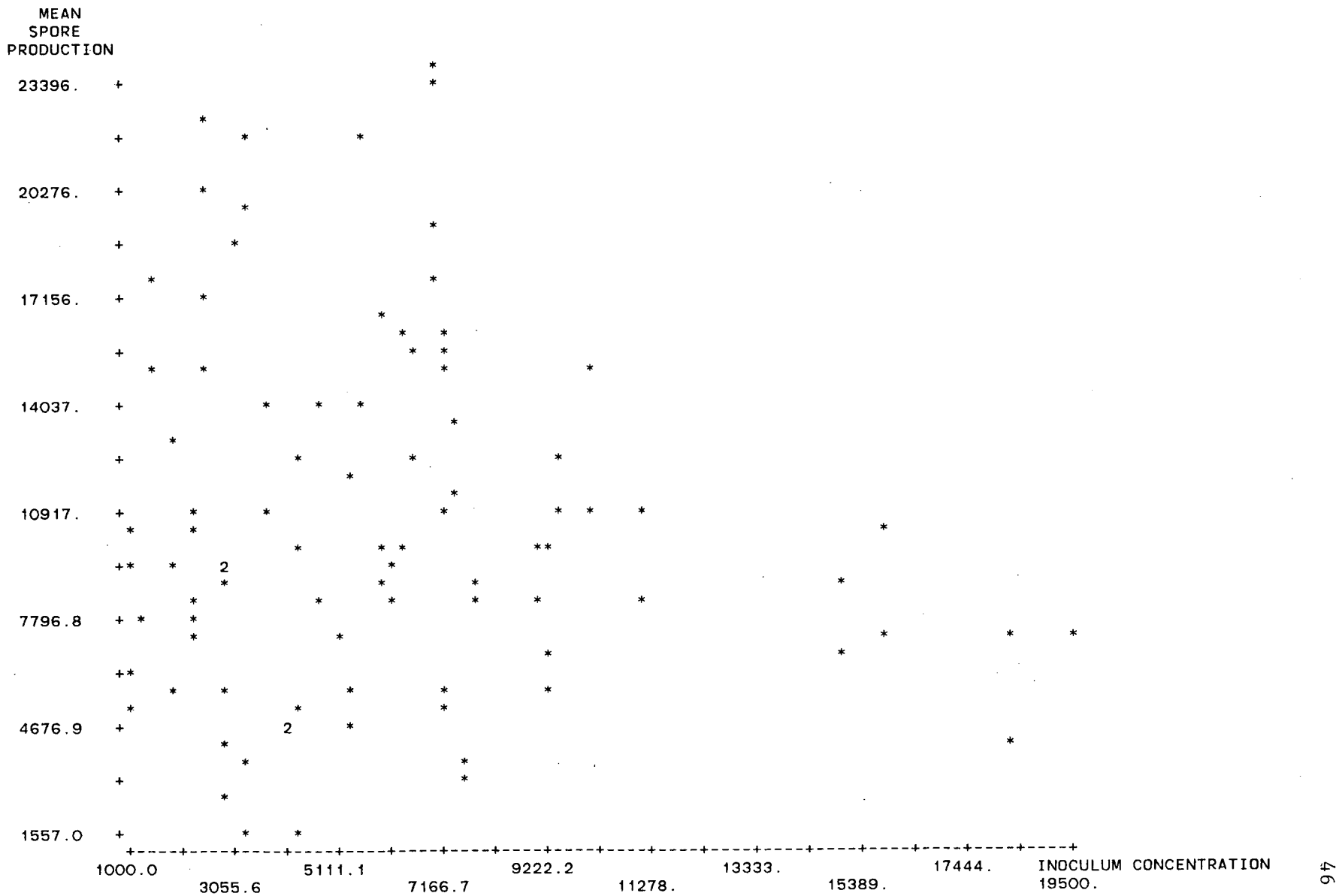
Three stems were stripped entirely of their leaves and the leaves were cut into halves for inoculation. The leaves were inoculated with several droplets of the spore suspensions of an isolate known to be virulent on that clone. The droplets were then spread all over the leaf halves with a paintbrush.

leaf	height* (cm)	length (cm)	width (cm)	pustules (number)	
1-1	2.2	8.0	2.5	0	
1-2	4.6	11.6	4.7	1	
1-3	7.2	14.9	6.5	8	**
1-4	10.5	15.5	5.7	8	
1-5	14.0	14.6	5.5	4	
1-6	18.0	13.8	5.9	1	
1-7	21.2	10.8	5.4	10	
1-8	24.8	11.5	5.2	10	
2-1	.2	5.2	1.8	0	
2-2	.5	5.5	1.9	0	
2-3	1.2	4.9	2.0	10	**
2-4	2.4	4.6	2.1	2	
2-5	4.7	4.8	1.9	5	
2-6	6.5	5.4	1.8	5	
2-7	7.8	4.3	1.7	10	
2-8	9.0	5.0	1.9	1	
2-9	10.2	5.0	1.9	12	
2-10	12.0	6.0	2.3	8	
3-1	1.0	7.5	3.9	0	
3-2	2.0	7.0	3.4	0	
3-3	2.3	8.2	3.5	7	**
3-4	4.5	9.2	4.3	8	
3-5	6.0	6.2	3.5	1	
3-6	7.2	8.1	3.8	25	
3-7	8.2	7.5	3.5	2	
3-8	9.8	8.0	4.0	5	
3-9	11.0	9.2	4.0	7	
3-10	13.2	7.2	3.4	8	

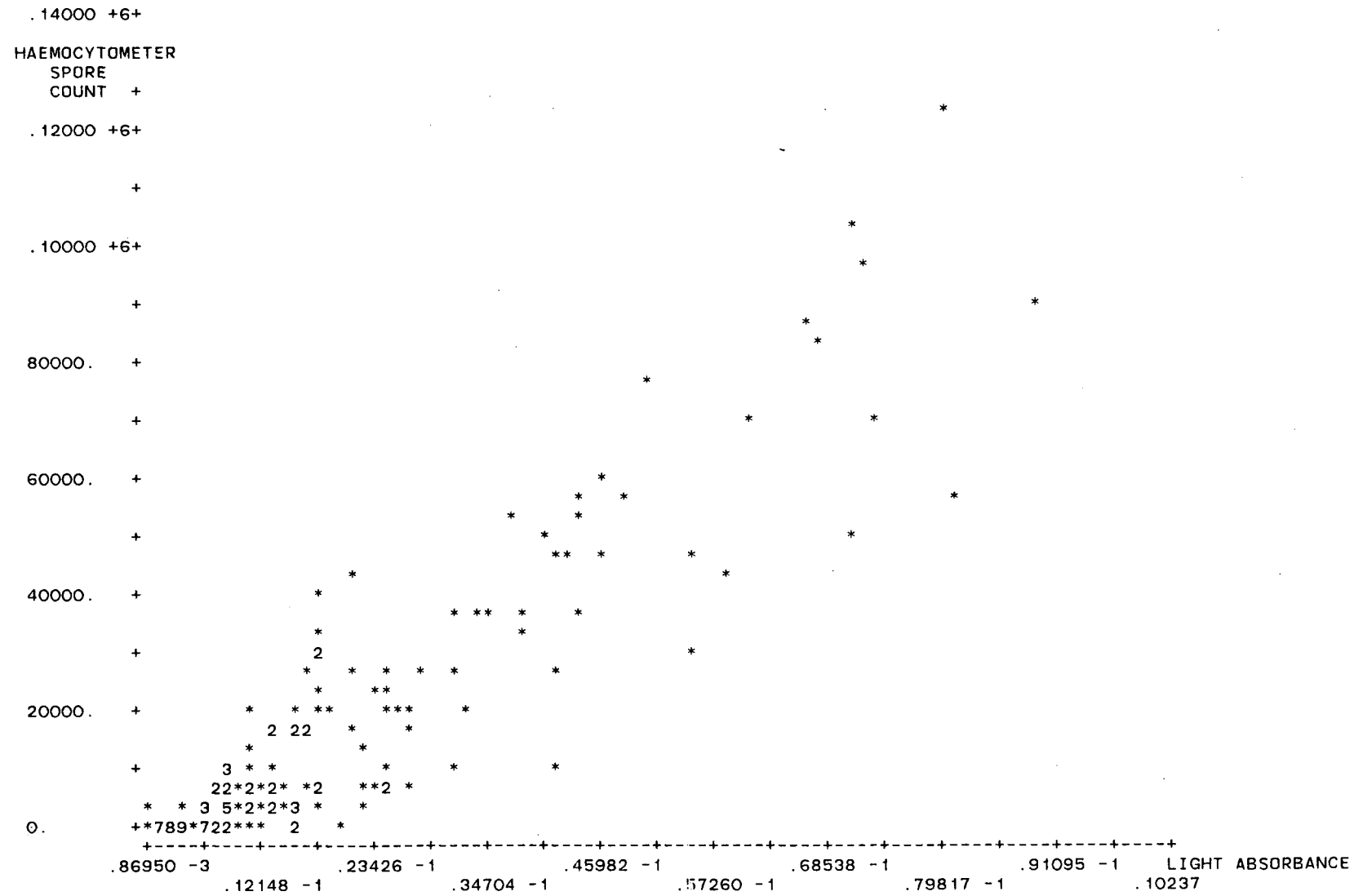
* height refers to distance from the top of the plant. and the smallest height means the youngest leaf. (e.g. leaf 1-1 was the youngest leaf on stem 1).

** This was judged to be the most recently expanded leaf based upon the length and width of the leaf. By pustule number, this leaf was also found to be the youngest or second youngest leaf susceptible to the rust.

APPENDIX D: Mean average daily spore production of each petri dish vs. inoculum concentration applied to i dish. The correlation is r=-.14, which is not significant.



APPENDIX E: Haemocytometer spore count vs. light absorbance of the same spore suspension.
This relationship was used to derive an equation for calculating spores from absorbance.
The derived equation: SPORES = 1,228,300 * ABSORBANCE - 7294 has an r=.91



APPENDIX F: ANALYSIS OF VARIANCE

1. Average spore production during twice the latent period for all specimens

SOURCE	D. F.	F-VALUE	F-PROB
Block	8	6.944	.0000
Treatment	139	2.357	.0000
Isolates	9	5.318	.0000
Clones	13	14.442	.0000
Interaction	117	.923	.6884
Error	1112		
Total	1259		

2. Average spore production for Vancouver Region specimens

SOURCE	D. F.	F-VALUE	F-PROB
Block	8	5.003	.0000
Treatment	76	1.885	.0001
Isolates	6	8.600	.0000
Clones	10	5.319	.0000
Interaction	60	.870	.7457
Error	608		
Total	692		

3. Pustule count over twice latent period

SOURCE	D. F.	F-VALUE	F-PROB
Block	8	6.693	.0000
Treatment	139	2.514	.0000
Isolates	9	8.160	.0000
Clones	13	17.293	.0000
Interaction	117	.851	.8673
Error	1112		
Total	1259		

4. Number of spores produced during twice latent period

SOURCE	D. F.	F-VALUE	F-PROB
Block	8	6.865	.0000
Treatment	139	2.341	.0000
Isolates	9	7.219	.0000
Clones	13	16.117	.0000
Interaction	117	.831	.8989
Error	1112		
Total	1259		

5. Latent Period (time in days from inoculation to symptoms)

SOURCE	D. F.	F-VALUE	F-PROB
Block	8	3.548	.0000
Treatment	139	1.825	.0000
Isolates	9	7.714	.0000
Clones	13	8.105	.0000
Interaction	117	.869	.8321
Error	1112		
Total	1259		

APPENDIX G: Analysis of variance for isolate-clone interaction where lowly correlated clones and isolates have been subject to analysis on U.B.C. MTS program *ANOVAR

```
          MODEL,SPORIN=A+B+C+AC+E
          LIMITS,2,9,2
*******   FACTORIAL DESIGN
*******   A1=Hebb A2=Gran C1=CAL C2=IMP
          DATA,-DAT
          RANDOM,B
          RENAME,BLOCK=B,ISOLATES=A,CLONES=C,INTERACTION=AC
          RANGE,(SOURCE=ALL),(SIGREQD=NONE),(CORREL=YES)
          OPTIONS,PRNTEMS
          INPUT,A(5,2),B(2,2),C(8,2),SPORIN(55,8)
```

ANALYSIS OF VARIANCE FOR AVERAGE SPORE PRODUCTION RATE

SOURCE	D.F.	F-VALUE	F PROB
ISOLATES	1	0.9022	0.3543
CLONES	1	3.1251	0.0864
INTERACTION	1	10.8899	0.0031 **
BLOCK	8	1.1114	0.3905
ERROR	24		
TOTAL	35		

	ISOL * CLONE	MEAN	STD ERROR	RANGE TEST
1.	Hebb * CAL	2324.000	168.444	A
2.	Hebb * IMP	454.889	40.725	B
3.	Gran * CAL	756.556	26.846	B
4.	Gran * IMP	1321.667	177.073	B A

** This was the only significant F-value (< .05), so a Duncan's Multiple Range Test was performed on these means of isolate-clone interaction. Means followed by the same letter (A or B) are not significantly different.

The conclusion can be made that elimination of closely correlated clones and isolates (with respect to disease severity) can lead to a significant isolate-clone interaction but without the more important significant differences between clones or between isolates.

APPENDIX H: CALCULATIONS FOR COMPONENTS OF VARIANCE

Begin with an analysis of variance with mean squares:

Source	degrees of freedom	mean square
blocks	8	9013334
isolates	9	6415802
clones	13	17423792
interaction	117	1206473
error	1112	1298330

Then calculate the expected mean squares (EMS):

EMS(blocks) = VAR(error) + N * A * C * VAR(blocks)

EMS(isolates) = VAR(error) + N * B * C * VAR(isolates)
+ N * B * VAR(interaction)

EMS(clones) = VAR(error) + N * A * B * VAR(clones)
+ N * B * VAR(interaction)

EMS(interaction) = VAR(error) + N * B * VAR(interaction)

EMS(error) = VAR(error)

Let the EMS values equal their mean square counterparts, and from the analysis of variance:

N is the number of replications 0
A is the number of isolates 10
B is the number of blocks 9
C is the number of clones 14

Then solve for the variances (VAR):

VAR(blocks) = (MS(blocks) - MS(error)) / N * B
= 55107
VAR(isolates) = (MS(isolates) - MS(interact)) / N * B * C
=41344
VAR(clones) = (MS(clones) - MS(interact)) / N * A * B
= 180192
VAR(interact) = (MS(interact) - MS(error)) / N * B
= 0
VAR(error) = MS(error) =1298330

VAR(total)= 1574973 (the sum of these variances)

Now calculating all the variances as a percentage of the total will give what is found in Table III.