SELECTION OF PARTIAL RESISTANCE FOR CROWN RUST (*Puccinia coronata* Cda.) RACE 264 IN OAT.

By

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FORWARD

This thesis is submitted in the form of original papers suitable for journal publications. The first section is a literature review presenting the theory and previous knowledge on this topic. The next two sections represent the body of the thesis (each is a complete manuscript). The last section is a general discussion and a synthesis of the major conclusions. This thesis format has been approved by the Faculty of Graduate Studies and Research, McGill University, and follows the conditions outlined in the Guidelines Concerning Thesis Preparation, section B.2 "Manuscripts and Authorship" which are as follows:

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Although all the work here is the responsibility of the candidate, the project was supervised by Dr. A.C. Kushalappa, Department of Plant Science, Macdonald Campus of McGill University. The first of the two manuscripts is co-authored by Dr. A.C. Kushalappa and Dr. D.E. Mather and the second manuscript is co-authored by Dr. A.C. Kushalappa. For consistency and convenience, all manuscripts follow the same format. The copies that will be sent to the respective journals, however, will follow the requirements of each journal. The first and second manuscripts will be submitted to the Canadian Journal of Plant Pathology and Phytopathology, respectively.

ABSTRACT

M.Sc. STEPHAN C. BRIERE Plant Pathology

SELECTION FOR PARTIAL RESISTANCE TO CROWN RUST *Puccinia coronata* Cda. RACE 264 IN OAT CULTIVARS AND BREEDING LINES

Nineteen cultivars and fourteen breeding lines were evaluated for partial resistance to crown rust *Puccinia coronata* race 264 under field and growth bench conditions. Multivariate statistical methods such as principal component and cluster analyses were employed to identify significant resistance parameters and to group oat genotypes with similar rust resistance characteristics. This involved two separate investigations. The first investigation consisted of two experiments, the first experiment was conducted under field conditions and the second experiment was conducted under growth bench conditions. In 1989 and 1990 the 33 oat genotypes were sown in 3.8 x 1.0 m field plots consisting of five rows each. A hill in the centre of each plot was inoculated with *P. coronata* race 264. Crown rust severity was visually estimated at weekly intervals, beginning at the first sign of disease, as the percent leaf area diseased. The results of the cluster analysis for both years were very consistent, with 5 and 6 cluster groupings respectively, for each year. The first cluster of each field trial identified the same 6 and 7 oat genotypes for both years. A simplified field screening procedure was devised using only the data from only flag leaves sampled at both Zadoks growth stages 53 and 85. In the second experiment the same 33 oat genotypes were grown in pots, placed on a growth bench and inoculated with *P. coronata* race 264 using an automated spray chamber. The disease parameters used to screen for partial resistance were mean latency period (MLP), total number of lesions per leaf (MAXLES), and lesion area. Very similar cluster groupings were obtained for both trials of the latter experiment which were comparable to those obtained in both trials of the field screening experiment. The second investigation employed 7 oat genotypes with known partial resistance characteristics from the previous investigation and were used in two further experiments. The first experiment was conducted under field conditions, the 7 oat genotypes were sown in 5 x 5 m plots, hill in the center was inoculated with *P. coronata* race 264. The percent leaf area diseased was estimated weekly after the onset of disease. This experiment had cluster groupings that were comparable with the results obtained from the previous investigation. The second experiment of this investigation was conducted under growth bench conditions. The disease parameters quantified were total sporulation/leaf, sporulation/lesion, MAXLES and MLP. Each trial had 3 cluster groupings with oat genotypes displaying high partial resistance located in the first cluster. The latter results were comparable to the previous experiment except that two oat genotypes with high partial resistance were grouped with oat genotypes with medium to low partial resistance. From both of the above investigations a group of oat genotypes with high partial resistance to *P. coronata* race 264 was obtained. These are OA 712-17, OA 712-33, Glen, Woodstock, QO 220.13, and QO 574.21. These oat genotypes are currently being used as parents in crosses in the Macdonald Campus of McGill University oat breeding program.

RESUMÉ

M.Sc. STEPHAN C. BRIERE Phytopathologie

SÉLÉCTION POUR LA RESISTANCE PARTIELLE AU CHAMPIGNON *Puccinia coronata* Cda. RACE 264 DANS DES CULTIVARS ET LIGNÉES D'AVOINES.

Dix-neuf cultivars et quartorze lignees d'avoines ont ete évalues quant a leur niveau de résistance partielle aux champignon *Puccinia coronata* race 264 au champ et sur banc de croissance La sélection de paramètres de resistance et de méthodes appropriees a également été effectué Des analyses multidimensionnelles comme les analyse en composantes principales et de groupement ont été utilisées pour sélectioner les paranètres de résistances important afin de grouper les génotypes d'avoine ayant des caractéristiques semblabes de résistance partielle à la rouille couronnée La première étude comprenait des expériences aux champ et sur banc de croissance ou les 33 génotypes d'avoine ont été testés Durant les étés de 1989 et 1990, les 33 génotypes d'avoine ont été semé dans des parcelles de 3 8 x 1 0m avec cinq rangs Des plants situés sur une butte au centre de chaque parcelle ont été inoculés avec *P coronata* race 264 La sévérité de la maladie sur 5 feuilles de 4 plants par parcelle a été estimee visuellement et exprimée en pourcentage de la surface foliare malade une fois par semaine debutant avec l'apparition de la maladie Les donnees des deux étés sont très consistantes, resultants en 5 et 6 différents groupements respectivement pour chaque essai Le premier groupe de chaque essai consistait en 6 et 7 génotypes d'avoine, les mêmes pour les deux essai En utilisent les résultats de la feuille étandard seulement, une méthode d'échantillonnage simplifier qui utilisent seulement la maladie sur les feuilles étandard aux stades de croissance Zadoks 53 et 85 a été établie Les mêmes 33 génotypes d'avoine ont été semé en pots, placés sur banc de croissance, et la première feuille a été inoculée avec *P coronata* race 264 utilisant un vaporisateur automatisé Les paramètres de résistance utilisés pour la sélection de la résistance partielle était, la période de latence moyenne (PLM), le nombres de lésions totales (LESTOT), et la surface moyenne des lésions Des groupements similaires ont été obtennus pour chaq'un des deux essais sur banc de croissance ainsi qu'au champ Sept génotypes d'avoine ont été choisis à partir de la dernière étude et ont été étudiés d'avantage dans une deuxième étude sur banc de croissance et au champ Les paramètres de résistance qui ont été quantifiés sur banc de croissance était la sporulation total/feuille, (SPOTOT), LESTOT, PLM, et sporulation total/lésion Les deux paramètres SPOTOT et LESTOT ont été analysés avec les analyses de composantes principals et de groupement pour obtenir 3 groups de génotypes d'avoine pour chaq'un des deux essais Le premier groupe incluait des génotypes d'avoine reconnu pour leurs haut niveau de résistance partielle Les mêmes sept génotypes d'avoine ont été testés en parcelle au champ de 5 x 5m dans lesquelles une butte au centre a été inoculés avec le *P coronata* race 264 L'echantillonnage des parcelles a été accomplı de la même façon que pour l'autre étude au champ Le résultat de l'analyse de cette expérience fut semblable aux résultats obtenus sur banc de croissance Les deux études ont donné un group de génotypes d'avoine avec un haut niveau de résistance partielle, OA 712-17, OA 712-33, Glen, Woodstock, QO 220 13 et QO 574 21 Ces génotypes d'avoine sont présentment utilisés dans des croisements d'avoine au Campus Macdonald de l'Université McGill

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Finally I would like to dedicate this thesis to my wife Cathy Blonk and also highly appreciate her total support during these very busy two years. I am very grateful for her patience during my absences on many evenings and weekends.

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INTRODUCTION

Crown rust of oats is an important fungal disease in North America and throughout the world. In Western Canada the disease is found in the prairie provinces of Manitoba and Saskatchewan. In Eastern Canada it is important in the areas to the west and south of western Québec and in parts of Ontario (Chong 1988b).

Crown rust of oats is caused by the fungus *Puccinia coronata* Cda. which is classified in the subdivision Basidiomycotina, order Uredinales The disease does not kill the oat plant but reduces the photosynthetic area available by covering the leaves with small orange pustules. The disease is important especially in the flag leaf where up to 80% of the assimilates go directly to filling the seed. Breeding for vertical resistance with specific resistance genes (vertical) is a feasible method to control the disease, but this type of resistance is quickly overcome by the pathogen.

An alternative to specific or vertical host resistance is the use of horizontal resistance. This type of resistance may also be termed partial, polygenic, field, adult plant, and durable resistance (Robinson 1989). When dealing with rust resistance this may also be termed slow rusting of the host. The term partial resistance is characterized by a reduced rate of epidemic build-up despite a high, susceptible infection type, by absence of large race specific effects and by durability (Parlevliet 1988). The latter term best fits the objectives of this research project, since it parallels our desires to be able to select susceptible oat genotypes with low disease accumulation over the growing season.

Politowski et al. (1978) working with *P. coronata* sought to find whether horizontal resistance (or dilatory resistance as they termed it) could be assessed among cultivars by using host yield, grain quality, and spore production parameters. These were done under both field and greenhouse conditions. They found that using yield and grain quality measurements for quantifying horizontal resistance was not practical if the methods used were not very precise. This may lead to one cultivar being rated tolerant when it actually is not. They also compared spore production under field conditions with that of disease parameters collected in greenhouse experiments. They found a good correlation between lower spore production in the field with fewer pustules per leaf and less pustule area per leaf in the greenhouse. They concluded that these epidemiological parameters were more accurate and practical in determining horizontal resistance than using yield and grain quality measurements.

Similarly, Singleton et. al. (1982) working with crown rust of oats, compared yield and disease parameters to screen for horizontal resistance. They concluded that yield and 250-kernel weight ratios did not clearly differentiate the tested cultivars for horizontal resistance as did the comparison of the area under the disease progress curve for each cultivar.

Shaner *et. al.* (1978), working with *P. recondita* on wheat used latency period, lesion size, and spore production to screen cultivars in the greenhouse. They ascertained that the latency period lagged 2-4 days for some cultivars, and lesion size tended to be smaller on slow-rusting cultivars. However, when comparing spore production on the

lesions of equal size on fast and slow rusting cultivars, they found no disparity between cultivars. Therefore the development of disease in slow-rusting cultivars was slower due to the combined effect of longer latent period and smaller lesion size. They suggested a quick method for the screening of slow rusting was to count the uredia on inoculated plants 8-9 days after inoculation. As this is the time where there is the greatest difference between slow and fast rusting cultivars. Futhermore a second inspection at 14 days after inoculation could be used to identify differences in infection efficiency and uredium size.

LITERATURE REVIEW

1.0 OAT PRODUCTION

Canada ranks among one of the largest oat producers in the world. In 1988 there was 1 137 000 ha of oats grown with a total production of 2 933 000 metric tonnes. In 1987 the value of the Canadian oat crop was 49 million dollars and in 1988 this value was 111 million dollars, an increase of 126% in just one year. In 1989 this figure is expected to rise again, during this same year Canada became the largest exporter of oats in the world (Agriculture Canada 1989).

In Québec in 1988 oats were grown on 121 000 ha with a total production of 284 000 metric tonnes. The value of the Québec crop in 1987 was 9 million dollars and in 1988 it was 15.5 million dollars, an increase of 72% over the previous year (Agriculture Canada 1989). This increase was attributed to the interest over the past few years of using oat bran to reduce cholesterol levels in human beings.

2.0 CROWN RUST OF OAT

2.1 Taxonomy

The crown rust fungus is classified in the Class Basidiomycetes; order Uredinales (Alexopoulos, 1979). Arthur (1962) classifies it in the family Pucciniaceae, that is

characterized by having well circumscribed telia and free or fascicled teliospores. It is classified in the genus *Puccinia* and species *coronata* and was first described by August Carl Joseph Corda (Cda.) in 1837 (Arthur, 1962). *Puccinia coronata* Cda. is characterized as having usually four digitate projections on the top of the teliospore.

2.2 Host Plants

In North America there are many plant species in the host range of *P. coronata*. This is divided in two host range types, which are termed pycnial-aecial and uredial-telial hosts (Simons, 1970).

In Canada the pycnial-aecial host associated with *Avena* spp. is *Rhamnus carthica* L. (European buckthorn). It was introduced from Europe to serve as a woody ornamental shrub and is now widely distributed in the wild (Chong 1988b). Over the world there are many other pycnial-aecial hosts in the genus *Rhamnus*.

The uredial-telial hosts for *P. coronata* are found in 72 genera and comprise more than 290 species (Simons, 1970). Most of these species are found in the sub-family Festucoideae within which tribes such as Aveneae, Triticeae, Agrosteae, and Festuceae are found.

2.3 Crown Rust Life Cycle

The fungus *P. coronata* is a heteroecious rust having *Rhamnus carthica* L. as the

major alternate host in Canada. The uredial-telial cycle of the pathogen occurs in *Avena* spp. and the pycnial-aecial occurs in *R. carthica.*

The pycnia develops on *R. carthica* in late May and the first aeciospores mature within a week. The aeciospores then infect the oat host in early summer after which uredia develop and the uredospore repeating cycle commences. The disease continues to spread upwards into the oat leaf canopy by wind and rain splash dispersal. As the infected leaves mature and senesce, telia are formed in and around the uredia, serving as the overwintering structure of the fungus (Simons, 1970). Teliospores are released from the telia in the early spring and germinate. Meiotic reduction occurs resulting in the formation of haploid basidiospores which will infect the young leaves of *Rhamnus.* The sexual recombination of the fungus takes place on the upper leaf surface of *Rhamnus* during spermatization where insects inadvertently transfer spermatia to the receptive hyphae (Alexopoulos, 1979). The dicaryotic state of the fungus is restored before the production of aeciospores, which infects the oat host to complete the cycle.

Conners and Savile (1943) showed that in Canada a few strategically located buckthorn bushes can start an epidemic over a relatively large area. Conners and Savile (1952) also confirmed that certain races usually developed from buckthorn in Eastern Canada. Other races in the area were presumed to come from the United States where they predominated.

Because *P. coronata* is a heteroecious rust, the alternate host plays an important

role in the overwintering and sexual recombination of the fungus. This heteroecious nature of the pathogen has contributed to the formation of many different races with varying degrees of virulence (Simons, 1970).

In Canada different patterns of virulence combinations of *P. coronata* occurs in the two major areas of western and eastern Canada. There are fewer virulance combinations of *P. coronata* in the western Canadian provinces of Manitoba and Saskatchewan than there are in eastern Canada which comprises exclusively the provinces of Québec and Ontario (Chong 1988a). In both these regions the number of virulence combinations of P. coronata have been rising over the years, but in eastern Canada this has occured more rapidly due to the large concentration of the alternate host *Rhamnus carthica* and the increased use of cultivars with durable resistance to *P. coronata*

2.4 Symptoms on Oat

The crown rust fungus usually develops primarily on the upper and lower surfaces of the oat leaf blades. Some lesions may also develop on the stem and on floral structures. The initial symptom after infection of oat leaves with the crown rust fungus is the appearence of expanding circular chlorotic areas followed by the formation of orange uredia that are circular to oblong in shape and average 5 mm in length within these chlorotic areas. There also maybe varying degrees of chlorosis encircling the uredia but this is dependant on the resistance of the oat genotype affected The uredia will begin to appear after a latency period of approximately 8 to 10 days. The uredia then erupt and release large amounts of bright orange uredospores. As the infected leaves mature and

senesce, black to dark brown coloured telia are formed in and around the uredia and remain covered by the epidermis. The telia may also form in rings around the uredia (Simons, 1970).

2.5 Factors Influencing Crown Rust Development

2.5.1 Factors Influencing Infection

Politowski (1975) determined the minimum and optimum dew period (hours of leaf wetness) needed for pustule formation of oat leaves by *P. coronata*. The minimum hours of dew period required for pustule formation was found to be 5 hours at 21°C. The optimum length of dew period for maximum infection was determined to be 16 hours at 21°C.

The temperature range required for optimum pustule development by *P. coronata* was determined by Politowski (1975). The results showed no germination occurred during a dew period at 4.5°C. A regression analysis of the data suggested the optimum temperature range required for pustule development to be 17 to 22°C.

A study to determine if light had any effect on pustule formation in oat by *P. coronata* was conducted by Politowski (1975). Oat and wheat plants inoculated with *P. coronata* and *P. graminis tritici* respectively, were placed in lighted and darkened compartments of a dew chamber set at 21°C for a period of 12 hours. The results for *P. coronata* demonstrated that there was no significant difference in the number of pustules

formed when the plants were incubated in the dark or light. This is contrary to the results obtained with *P. graminis tritici* where the mean number of pustules was 16 in light and 1 in dark. Therefore the presence or absence of light had no significant effect on pustule formation by *P. coronata.*

2.5.2 Factors Influencing Latency Period

Temperature has been shown to have an influence on the latent period of *P. coronata* (Simons, 1970). Maryland, as quoted by Simons (1970), showed that the latency period ranged from 20 days at 5-9°C to 6 days at 17-21°C. The levels of incomplete resistance can vary the length of the latency period in different cultivars with the same physiologic rust race (Simons, 1970).

In a study with 5 host genotypes of *Avena sterilis* and the oat cultivar Fulghum that were inoculated with 5 isolates of *P. graminis* f. sp. *avenae*, it was determined that the host genotypes did not differ in their latency period lengths despite differences in colony size (Parlevliet, 1979). The latter suggests that for *P. graminis* f. sp. *avenae* the latency period is not an important resistance parameter for the screening of oat cultivars to this fungus, but this may or may not apply to *P. coronata.*

2.6 Economic Importance

Crown rust of oat has reduced yield in some years as much as 30% in certain areas of the United States (Simons 1970). This yield decrease is in the form of lighter grain weight, which in turn leads to reduced grain quality. The latter is very important

when the grain is grown for human consumption.

2.7 Disease Control

2.7.1 Resistance

Race specific or vertical resistance is presently the only feasible method of control for crown rust of oat. This type of resistance in oat was reported first in 1927 when the susceptible Scotch Potato oat and the resistant Red Rustproof oat cultivars were crossed (Simons, 1984). This type of resistance is still widely used but has major limitations because the oat gene pool is not infinite and new rust races quickly develop resistance to newly introduced *Pc* genes.

The failure of specific resistance sparked interest in breeding for nonrace specific or horizontal resistance. This was first noticed in 1920 when segregating progenies from a cross between a resistant and a susceptible oat cultivar showed that multiple factors were responsible for the resistance (Simons, 1984). It has been shown that the resistance of the cultivar Red Rustproof was controlled by a small number of genes showing slight partial dominance for susceptibility. Heritability was high, with a broad sense value of 87%, making selection for this resistance possible (Luke, 1975). This type of resistance is more promising as a control strategy for crown rust because it is not race specific and will reduce the pressure on the pathogen to develop new races.

Resistant cultivars which have new resistance genes (*Pc* genes) from other *Avena*

spp. have been backcrossed into existing cultivars to provide complete vertical resistance to existing races of *P. coronata* (Simons 1985). This specific or vertical resistance is quickly broken down by the pathogen due to hybridization and mutations, and has led to "boom and bust" cycles of rust resistance. The resistance gene *Pc39* introduced into the cultivar Woodstock provided specific resistance to the current virulence combinations of *P. coronata* present at the time. However Chong (1988b) stated that following the introduction of Woodstock in 1982 there has been much selection pressure on the pathogen to overcome the resistance combinations which included the gene *Pc39*. In 1985 one isolate of *P. coronata* with a virulence combination including *Pc39* was found to be virulent on the cultivar Woodstock (Chong, 1986). In 1987 55% of the isolates of *P. coronata* in the rust population were virulent on the gene *Pc39*. By 1988 this value had increased to 87% (Chong and Seaman, 1990).

The genes *Pc38* and *Pc39* together have provided specific resistance to the pathogen when they were introduced into the genome of the cultivar Dumont, which is adapted to western Canada. The cultivar Newman which has the *Pc38* gen was developed by backcrossing the genes *Pc38* and *Pc39* from Dumont. During the summer of 1989 I observed here at the Macdonald Campus of McGill University Lods Agronomy Research Station that new virulence combinations of *P. coronata* were appearing on the cultivar Newman. Therefore this again shows that the effort to develop cultivars with durable resistance to crown rust is an exercise in futility.

2.7.2 Multilines

The term multilines, is applied to cultivars that are composed of two or more breeding lines. Politowski and Browning (1978) showed that multiline cultivars had significantly less disease than susceptible cultivars and only slightly more than resistant cultivars. Simons (1984) suggested that the mechanism by which multilines worked was that a portion of the inoculum produced will be effective only against a fraction of the oat population. This will ultimately slow the rate of epidemic buildup of the pathogen. A given resistant plant in the multiline also will pose as a physical barrier against complete spore dispersal to susceptible plants.

2.7.3 Eradication of *Rhamnus*

Recommendations for eradication of *Rhamnus* were published as early as 1886 but in 1887, this recommendation was not endorsed and in many areas this was unfeasible. Since this initial recommendation, some educational programs for farmers on the importance of buckthorn and the removal of buckthorn hedges have been beneficial in reducing the disease in some limited areas such as Iowa (Simons, 1984). This method of control is not feasible in Canada due to the widespread occurrence of buckthorn bushes in the wild.

2.7.4 Planting Date

Simons (1984) determined that the effect of *P. coronata* on oat yield and quality increased significantly when the planting date was delayed and was more pronounced in highly susceptible cultivars. Early-maturing oat cultivars are less damaged by crown rust

than are late-maturing cultivars.

3.0 MULTIVARIATE ANALYSIS FOR GROUPING CULTIVARS WITH SIMILAR DISEASE RESISTANCE CHARACTERISTICS

3.1 Principal Components Analysis

Factor analysis is a group of statistical analyses that are concerned with the identification of structure within a set of observed variables of which principal components analysis (PCA) is one such technique. PCA transforms the original set of variables into a smaller set of linear combinations that account for most of the variance of the original set. The major use of PCA is to compute factors (principal components) so as to explain as much of the total variation in the data as possible with as few of these factors as possible (Dillon and Goldstein, 1984).

The principal components are extracted so that the first principal component (PC_1) accounts for the largest amount of the total variation in the data. The PC_1 is a linear combination of the observed variables where the weights (eigenvalues) have been chosen to maximize the ratio of the variance of PC_1. The second principal component (PC_2) is the weighted linear combination of the observed variables which is uncorrelated with the first linear combination and accounts for the maximum amount of the remaining variation not already accounted for by PC_1 and so on until all of the original variation is accounted for. It is possible to extract as many principal components as there were original variables but the goal is to reduce the number of principal components that account for most of the

variation (Dillon and Goldstein, 1984).

PCA has been widely used in ecology and systematics but recently has been applied successfully to plant pathology (Hau and Kranz, 1990). The latter authors have shown many instances where PCA has been used successfully in plant pathology, the first being Analytis (1973) who used PCA to separate the effects of two major weather variables in predicting apple scab. More specific examples of the use of PCA in disease resistance work has been with Jeger (1980), who analyzed a total of 11 components of partial resistance of *Septoria nodorum* on 41 wheat genotypes. The PCA found four eigenvectors from the correlation matrix that could be interpreted as the toxin action of the fungus, reproduction, growth and establishment.

Lebeda and Jendrulek (1987, 1988) used PCA to evaluate for genetic similarity among 220 lettuce cultivars in the host-pathogen relationship with *Bremia lactucae*. The authors quantified the following epidemiological parameters; the percentage of infected plants, number of infected leaves per plant, degree of infection and the calculation of r and k. The eigenvectors were calculated from the correlation matrix and plotted onto PC_1 vs. PC_2 plot. The authors then projected the cluster analysis results onto the two planes of the PCA plot and pointed out that this method was as good as the minimum spanning tree for displaying clusters of similar cultivars.

Recently, Anderson *et al.* (1990) used PCA to illustrate the correlative nature of parameters of the multiple disease complex of peanut in the assessment of resistance in

55 peanut genotypes. The resistance parameters used in the PCA were collected in greenhouse experiments and were then compared to determine which ones were the most representative of the previous field screening. The results showed that lesion size, sporulation, and the percentage of sporulating lesions were more closely associated with the disease ratings in the field.

3.2 Cluster Analysis

Cluster analysis (CA) is a classification technique that is used to investigate the structure of a given data set with the intention of dividing its objects into a number of groups or clusters so that the objects within a group are as similar as possible and the objects of different groups remain distinct (Hau and Kranz, 1990). The objects in the original sample is characterized by several variables that may be of nominal, ordinal or metric scale. The results of the CA are usually graphically displayed as minimum spanning trees and dendrograms or as projection of clusters on a plot of two important principal components (Hau and Kranz, 1990).

The first instance of the use of CA in plant pathology was by Kranz (1968) who measured 10 quantitative elements of disease progress curves of 40 different pathosystems so as to find epidemiological patterns. He found that the original 40 pathosystems could be classified in 12 groups of disease progress curves. A more specific example with using CA in an attempt to group host genotypes for their disease resistance characteristics is the work of Thompson and Ress (1979). They have classified 45 wheat cultivars into seven clusters based on the assessment of stem rust severity on six occasions. These clusters

mirrored the resistance reaction of the host plants, and were more closely related to the areas under the disease progress curves than the apparent infection rate.

Lebeda and Jendrulek (1988) attempted to group 26 lettuce cultivars for their resistance reactions to *Bremia lactucae* expressed as disease proportion for six sampling dates. Their CA gave them seven clusters and they stated that CA was more suitable for the description of the field resistance of the cultivars than using the apparent infection rate.

4.0 OBJECTIVES OF THE PROPOSED RESEARCH

The objectives of this research were first to evaluate nineteen oat cultivars and fourteen breeding lines for partial resistance to *P. coronata* race 264 based on their reactions to disease parameters such as the cumulative proportion of leaf area diseased, mean latency period, sporulation characteristics, and lesion area. Secondly to identify the significant resistance parameters useful for screening for crown rust and to develope a simplified screening method for the selection of oat genotypes with high levels of partial resistance under field and controlled environmental conditions.

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II. SCREENING FOR PARTIAL RESISTANCE TO CROWN RUST (*Puccinia coronata* Cda.) RACE 264 IN 33 OAT CULTIVARS AND BREEDING LINES

INTRODUCTION

Crown rust of oat caused by the fungus *Puccinia coronata* Cda. is an important foliar pathogen of cultivated oat in all major growing areas (Fig. 1a). In Canada two important *P. coronata* populations exist; the first is located in western Canada in the provinces of Manitoba and Saskatchewan; the second comprises Ontario and Québec (Chong, 1988a). In the latter, the population develops new races quickly due to the larger concentration of the alternate host *Rhamnus carthica*, on which the sexual recombination of the fungus occurs. This combined with the fact that completely resistant cultivars increase the selection pressure on the pathogen to evolve new races has lead to the accelerated breakdown of resistance to *P. coronata* in eastern Canada which has caused "Boom and Bust" cycles of rust resistance. An example of this is with the cultivar Woodstock which has resistance gene *Pc39*. It was highly resistant to crown rust when first released in 1982. By 1985 there was one virulent combination of *P. coronata* present on Woodstock (Chong, 1988b).

An alternative to race specific (vertical) resistance is the use of partial (horizontal) resistance. Partial resistance is characterized by a reduced rate of epidemic build-up despite a high, susceptible infection type, by absence of large race specific effects, and

Fig. 1: A, Crown rust (*Puccinia coronata* Cda.) uredia on the flag leaf of the cultivar NZ-3. B, Inoculation of oat plants in the automated spray chamber. C, Uredospore collection apparatus setup on the top of a growth bench. D, Closeup view of a ventilated polyethylene uredospore collection tube.

Fig. 1

by durability (Parlevliet, 1988). In order to breed new cultivars with high levels of partial resistance it is important to have parental genotypes of known levels of partial resistance and simple selection methods under both field and controlled environmental conditions.

Quantitative disease parameters have been shown to be quite effective to screen for partial resistance as opposed to yield parameters which have been proven to be unreliable (Politowski and Browning, 1978; Singleton, 1982). Application of multivariate analyses such as principal component and cluster analyses have proven to be valuable for selection of disease parameters and grouping of plant genotypes of similar resistance characteristics (Jeger, 1980; Lebeda and Jendrulek, 1987; Anderson, 1990).

To devise a simplified sampling method to screen for partial resistance in oat to crown rust under field conditions, it is important to reduce the number of leaves to be sampled and the number of sampling dates. Since the flag leaf of cereals is known to contribute over 80% of the assimilates that go to the filling of the grain, this leaf should be the most important one to be sampled (St-Pierre and Gendron, 1982). It is also important that the disease progress in the flag leaf be similar to that obtained from using the top five leaves in order to adequately assess the partial resistance of each oat genotype. Therefore the CPLAD data obtained for the flag leaf (CPLADF) for all genotypes was analyzed separately and the results were compared to those obtained from the analyses using the CPLADT values.

MATERIALS AND METHODS

Field Study

The field experiment of this study was conducted over two growing seasons (1989, 1990) at the Emile Lods Research Station of McGill University in Ste. Anne de Bellevue, Québec. Nineteen cultivars and fourteen oat breeding lines (Table 1) were sown in 3.8 x 1.0m plots in a randomized complete block design with three replicates. Each plot had 5 rows with 0.20m spacing and was seeded at a rate of 210 seeds/m and had 0.20m spacing between plots and 1.6m spacing between blocks. A hill in the centre of each plot was seeded with the oat genotype NZ-3 to serve as an inoculated spreader for disseminating the disease within each plot.

The race of *Puccinia coronata* selected for all the field and growth bench experiments reported here was race 264, which is also known as Winnipeg rust culture CR13 (Chong, personal communication). The virulence combination (effective/ineffective host Pc genes) for this race of *P. coronata* is as follows; (35,38,50,56,58,59,61,62,63,64,67,68/39,40,45,46,48,54,55,60). Race 264 is a common race of *P. coronata* in Québec and Ontario and since virulence to the gene Pc39 has increased to over 85% in 1988, this race is useful for testing for partial resistance in oat cultivars and breeding lines in south-western Québec (Chong, personal communication).

Table 1. List of oat cultivars and breeding lines used in the field and growth bench experiments.

Cultivars and Breeding Lines used in Field and Growth Bench Experiments					
Glen	(A)	QO 224.5	(L)	QO 573.10	(W)
Fidler	(B)	Kamouraska	(M)	QO 574.21	(X)
OA 712-17	(C)	Baldwin	(N)	QO 576.11	(Y)
OA 712-33	(D)	Nova	(O)	QO 576.27	(Z)
Oxford	(E)	Shaw	(P)	QO 576.39	(1)
QO 220.13	(F)	Laurent	(Q)	QO 565.7	(2)
QO 220.9	(G)	Newman *	(R)	QO 565.14	(3)
Woodstock	(H)	Dorval	(S)	Yamaska	(4)
Capital	(I)	Lamar	(T)	Lasalle	(5)
Marion	(J)	Tibor	(U)	Donald	(6)
Manic	(K)	QO 573.5	(V)	NZ-3	(7)

* This cultivar was omitted in the growth bench experiment due to its complete resistance to *P. coronata* race 264.

Note: The letter or number in brackets identifies the oat genotypes in the PCA plots (Figs. 2,3,4 and 5).

Ten plants from each hill were syringe inoculated at the third leaf stage with 1mL of a uredospore suspension (approximately 100 000 uredospores/mL) of *P. coronata* race 264. Commencing at first sign of disease four plants per plot were randomly selected from which five leaves per plant were nondestructively sampled thesc were the flag leaf and the 4 leaves preceding it. Crown rust severity was visually estimated as the percent leaf area diseased (PLAD) at weekly intervals until the senescence of the flag leaf. The Distrain computer program (Tomerlin, 1988) was used as a training to visually estimate disease severity on oat leaves. The Zadoks decimal code for growth stage for cereals (Zadoks, 1974) was assessed for each oat genotype on each sampling date.

The data on PLAD per leaf were converted into cumulative proportion of leaf area diseased (CPLAD) for each sampling date and averaged per plot using the Dispar computer program (Kushalappa and Carisse, 1989). This computer program is used to calculate various epidemiological disease parameters such as from disease severity data. The CPLAD values for all 5 leaves (CPLADT) and for the flag leaf alone (CPLADF) were then averaged for each plot. The mean CPLADT and CPLADF values for each sampling date (variables) for each oat genotype were then used in the statistical analyses.

Controlled Environment Study

The experiment of the controlled environment study was conducted twice on a growth bench at the Macdonald Campus of McGill University Phytorium. The same oat

genotypes listed in Table 1 were used in this study, except for the cultivar Newman which was omitted because it has the resistance gene *Pc38* which gives it complete resistance to race 264. Each oat genotype was sown in 10cm pots at the rate of 8 seeds/pot and placed on a growth bench set at 21°C in a completely randomized design with 3 replicates. When the first leaf was completely expanded the seedlings were thinned to 4 plants/pot and subsequently inoculated in an automated spray chamber (custom built by Research Instrument Mfg. Co. Ltd) with the leaves held in a horizontal position by modified paper clips to maximize spore deposition. The suspension of freshly collected *P. coronata* race 264 was adjusted to 100 000 spores/mL and was sprayed at a nozzle speed of 1.2 km/hr and pressure of 175 kPa (Fig. 1b). The pots were then watered and enclosed in plastic bags and sprayed with distilled water to provide the wetness required to induce infection. The bagged pots were then incubated on a growth bench for 48hr, after which the bags were removed.

Commencing on the 7th day after inoculation, the number of sporulating uredia were counted daily on all four inoculated leaves from each pot until all sporulating uredia had appeared (on day 15). The mean latency period (MLP) was calculated by using the equation (Eq. 1) developed by Shaner (1978) for each leaf as an average per plant per pot.

[1]

$$MLP = \sum p_i T_i$$

Where MLP is mean latency period

p_i = the proportion of maximum pustules formed on the i^{th} day after inoculation.

T_i = the total number of days since inoculation until the i^{th} day.

On the 14th day a leaf from each pot was randomly selected and the length and width of five randomly selected uredia were measured using a dial micrometer. Using the latter uredia length and width measurements and assuming that the uredia were rectangular in shape, the average lesion area (LSAREA) per pot was calculated. The maximum number of lesions (MAXLES) was determined by taking the total number of uredia present on the 15th day on all four leaves per pot and expressing the data as an average per pot. The MLP, MAXLES and LSAREA were calculated and the average per replicate for each oat genotype was used in the statistical analyses to group cultivars.

Data Analysis

Principal component analysis (PCA) was used in computing principal components to explain as much of the total variability among the oat genotypes as possible with as few principal components as possible, and to verify the weight of each variable on each principal component (Dillon and Goldstein, 1984). The variables used in the PCA were

the mean CPLADT and CPLADF values of each oat genotype for the field experiment, and the mean MLP, MAXLES and LSAREA values of each oat genotype for the growth bench experiment. The results from the PCA is reported here in two ways. Firstly the proportions of the first two principal components (PC_1 and PC_2) are represented as percentages of the total and the weights of eigenvectors for PC_1 and PC_2 are represented as a eigenvalues.

The second representation of the PCA is by placing each oat genotype on a PC_1 against PC_2 plot and by superimposing the results from the cluster analysis. To facilitate the plotting of the oat genotypes in the PCA plots an identification character (letter or number) was given to each oat genotype (Table 1) to reduce the physical space of each one in the plot and therefore reducing any overlapping of genotypes. This graphical method used by Lebeda and Jendrulek (1988) of superimposing groupings from the cluster analysis onto the PCA plot has been used. They proposed that this method is as good as the minimum spanning tree to display clusters of plant genotypes with similar disease resistance traits. The minimum spanning tree was found to be superior to the dendrogram for graphical presentation of similarities among varieties (Hau and Krantz, 1990). The PROC PRINCOMP procedure of the SAS© system was used to carry out the analysis (SAS, 1987). The variables used in the growth bench study were standardized and the PCA was preformed on the correlation matrix with a variance of one, because the original values were not on the same scale of measurement.

Cluster analysis (CA) was used to group oat genotypes based on the disease parameters which were quantified in the field experiments (CPLADT and CPLADF) and growth bench experiments (MLP, MAXLES and LSAREA). The CA method used was the unweighed pair-group method using arithmetic averages which in the SAS© system is equivalent to PROC CLUSTER - METHOD AVERAGE (SAS, 1987). The data fitted best as an analysis of objects (Q-analysis), and the data matrix was standardized in the growth bench study before analysis to remove the arbitrary effects due to the different scales of measurements of the variables. The CA results are shown graphically by using a dendrogram (also known as a cluster tree) which displays the paired oat genotypes in clusters, the average distance between clusters, and the coefficient of determination (R^2) at the place where the tree was cut to form a classification.

RESULTS

Field Study Based on Five Leaves Sampled

The field plots were sampled for disease severity a total of 7 times in the 1989 field trial and 5 times in the 1990 field trial. In the 1989 field trial the first sampling date was not included in the analysis because disease was present in only one individual oat plant sampled. The Zadoks growth stage (ZGS) for cereals was assessed for each oat genotype at every sampling date. This was then expressed as an average over all

genotypes for each sampling date (Table 2). This was done to select a suitable growth stage for a simplified screening method. When comparing the averaged ZGS for each sampling date over both years of field trials, parallel ZGS for both years can clearly be seen (Table 2).

The disease progress curves of the oat genotypes from the field experiments are expressed as a mean disease progress curve for each cluster grouping obtained from the dendrogram of the CA. This was done to compare the different characteristics of the disease progress curves of dissimilar clusters of oat genotypes which display similar resistance characteristics. These disease progress curves are based on average CPLADT values for each oat genotype for the 1989 and 1990 field trials and averaged within each cluster grouping (Figs. 2a and 3a). In Figures 2a and 3a the disease progress curves of clusters of oat genotypes with final CPLADT values below 0.10 had small slopes which denotes a much reduced rate of disease development as compared to the other cluster groupings in the graphs.

Principal Component and Cluster Analyses of the Field Experiment Results Based on Five Leaves Sampled

The results from the PCA and CA for both trials of the field experiment are shown in Figures 2 and 3 respectively. The principal components PC_1 and PC_2 from the PCA of

Table 2. Various sampling dates (SD) and corresponding averaged Zadoks growth stage (ZGS) values for both the 1989 and 1990 field experiments.

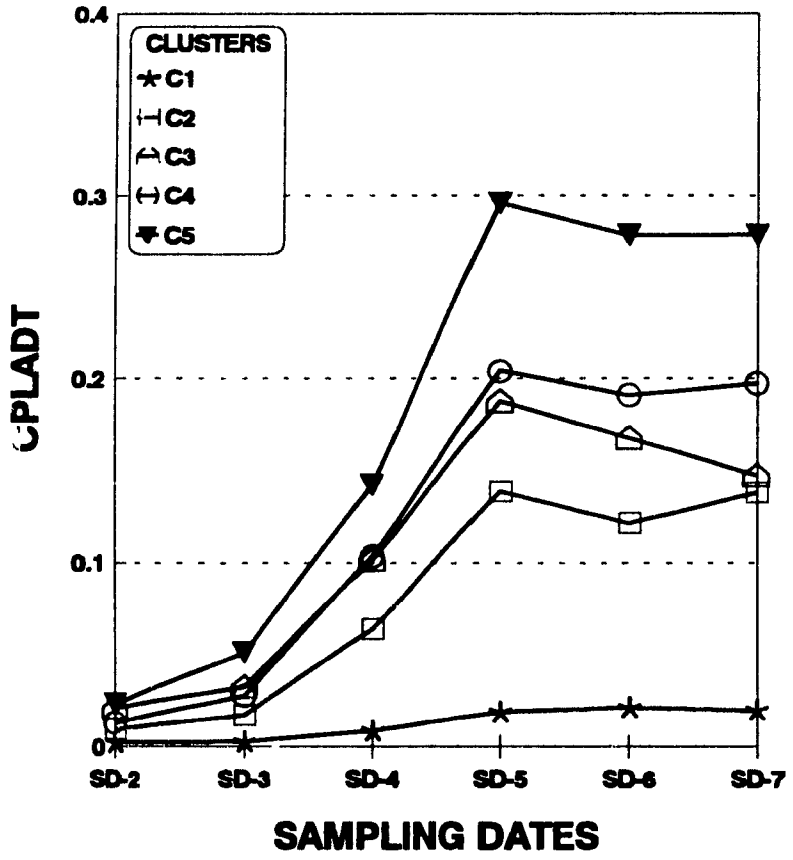
1989 FIELD TRIAL		1990 FIELD TRIAL	
SAMPLING DATE	**ZGS**	**SAMPLING DATE**	**ZGS**
SD-2	42	SD-1	43
SD-3	**53**	**SD-2**	**51**
SD-4	67	SD-3	69
SD-5	79	SD-4	77
SD-6	79	**SD-5**	**85**
SD-7	**85**	----	----

Note: The first sampling date (SD-1) was omitted because disease was only present in one individual plant. The sampling dates and growth stages shown in bold characters were those used in the simplified sampling method.

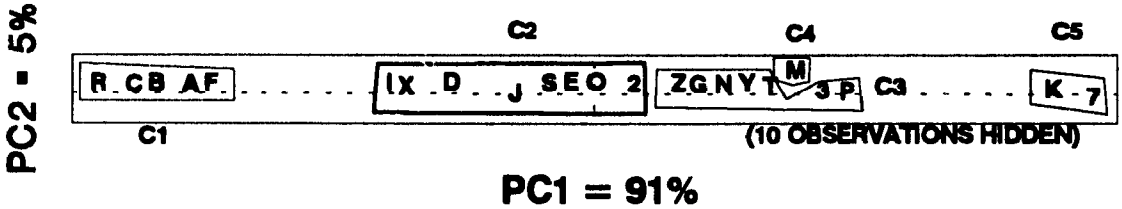
the 1989 field trial explained 91% and 5% respectively of the original variation attributed to the CPLADT variables on each of the six sampling dates. The eigenvectors obtained from the original CPLADT values for all six sampling dates were almost identical in

Fig 2: A. Disease progress of *P. coronata* race 264, expressed as the cumulative proportion of leaf area diseased (CPLADT) based on all 5 leaves sampled for oat genotypes within each of 5 clusters during the 1989 field trial. B. The plot of PC_1 v. PC_2 showing the location of each oat genotype in respect to each other and the results of the cluster analysis are superimposed onto the plot equally showing the distances between the cluster groupings relative to each other. C. The dendrogram of the CPLADT values for the sampling dates SD-2 to SD-7 for each oat genotype (R^2=0.87).

2A


CLUSTERS
C1
C2
C3
C4
C5
0.4
0.3
0.2
0.1
0
CPLADT
SD-2
SD-3
SD-4
SD-5
SD-6
SD-7
SAMPLING DATES

2B


PC2 = 5%
C1
C2
C4
C5
C3
(10 OBSERVATIONS HIDDEN)
PC1 = 91%

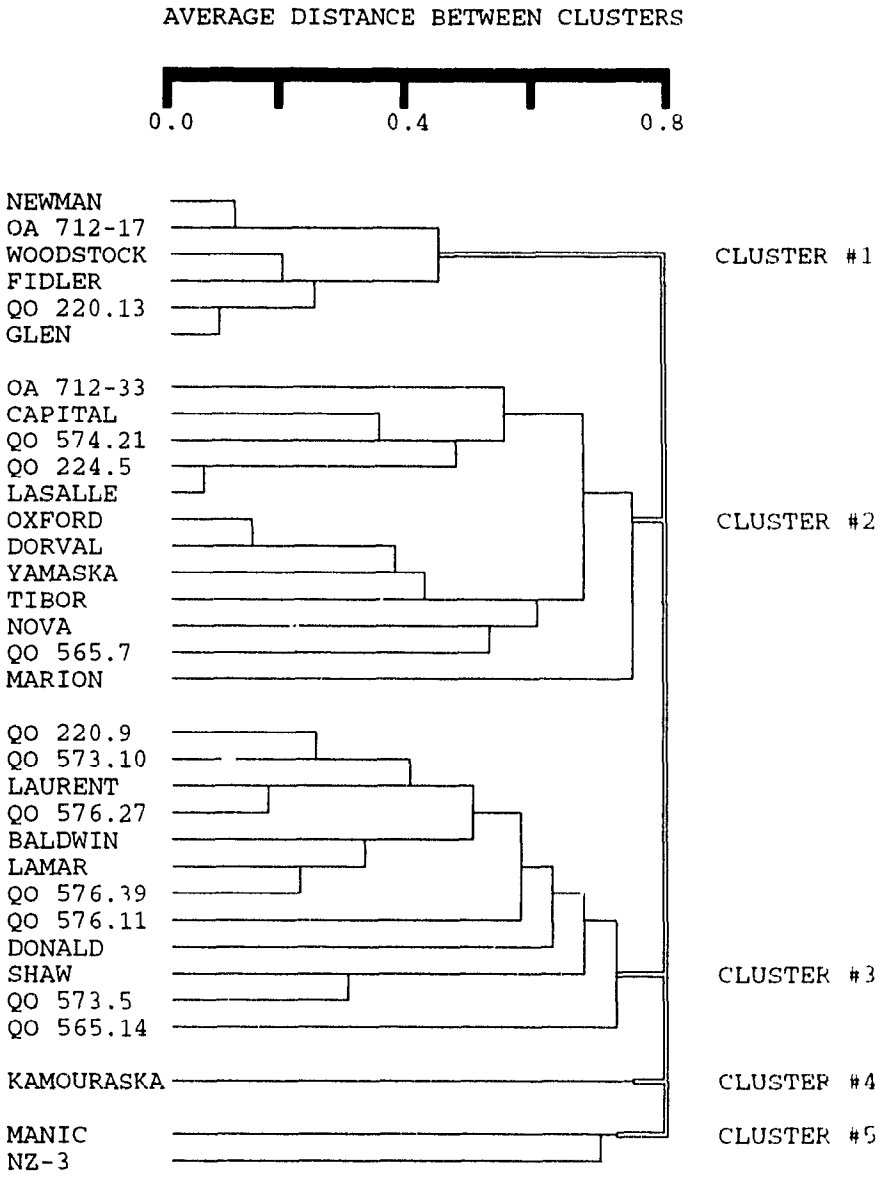
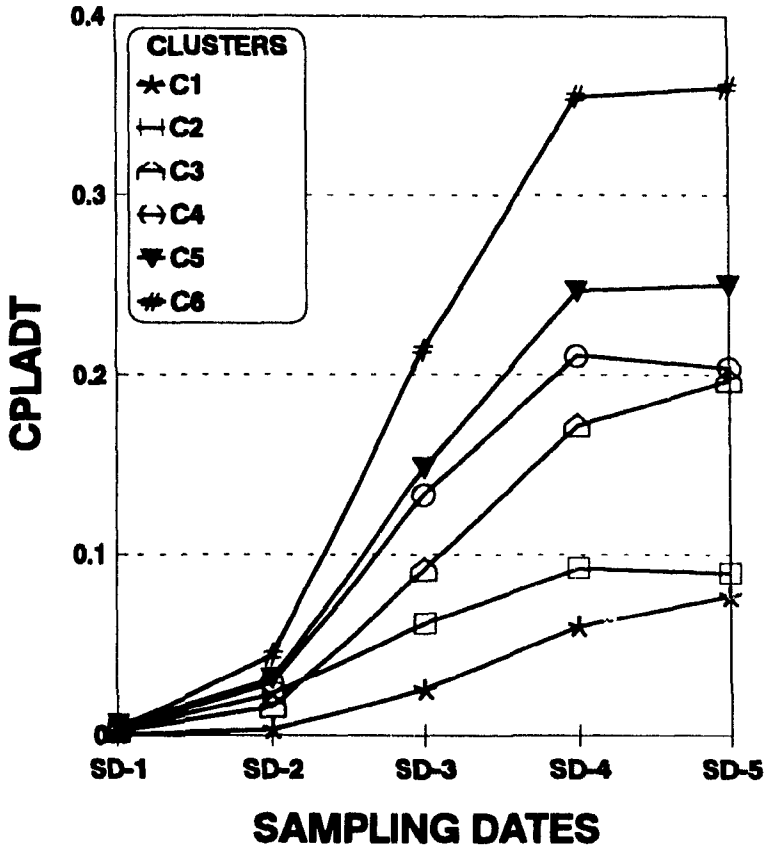
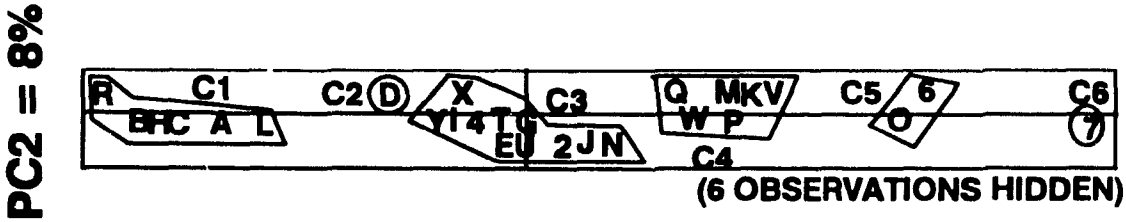

AVERAGE DISTANCE BETWEEN CLUSTERS
0.0
0.4
0.8
NEWMAN
OA 712-17
WOODSTOCK
FIDLER
QO 220.13
GLEN
OA 712-33
CAPITAL
QO 574.21
QO 224.5
LASALLE
OXFORD
DORVAL
YAMASKA
TIBOR
NOVA
QO 565.7
MARION
QO 220.9
QO 573.10
LAURENT
QO 576.27
BALDWIN
LAMAR
QO 576.39
QO 576.11
DONALD
SHAW
QO 573.5
QO 565.14
KAMOURASKA
MANIC
NZ-3
CLUSTER #1
CLUSTER #2
CLUSTER #3
CLUSTER #4
CLUSTER #5

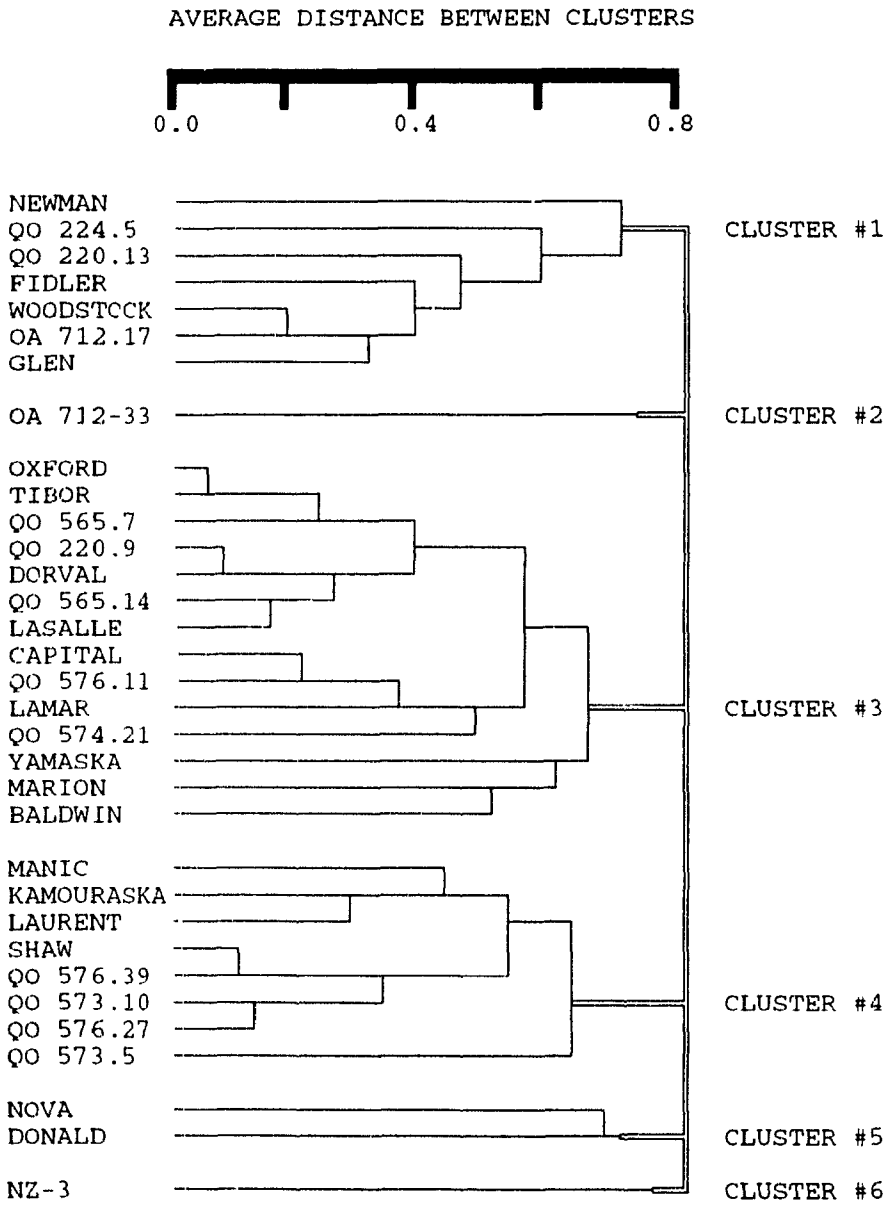
Fig 3: A. Disease progress of *P. coronata* race 264, expressed as the cumulative proportion of leaf area diseased (CPLADT) based on all 5 leaves sampled for oat genotypes within each of 6 clusters during the 1990 field trial. B. The plot of PC_1 v. PC_2 showing the location of each oat genotype in respect to each other and the results of the cluster analysis are superimposed onto the plot equally showing the distances between the cluster groupings relative to each other. C. The dendrogram of the CPLADT values for the sampling dates SD-1 to SD-5 for each oat genotype (R^2=0.83).

3A


CLUSTERS
C1
C2
C3
C4
C5
C6
CPLADT
0.4
0.3
0.2
0.1
0
SD-1
SD-2
SD-3
SD-4
SD-5
SAMPLING DATES

3B


PC2 = 8%
R
C1
BHC A L
C2
D
X
Y I 4 T G
EU 2 J N
C3
Q MKV
W P
C4
C5
6
O
C6
7
(6 OBSERVATIONS HIDDEN)
PC1 = 85%


AVERAGE DISTANCE BETWEEN CLUSTERS
0.0
0.4
0.8
NEWMAN
QO 224.5
QO 220.13
FIDLER
WOODSTOCK
OA 712.17
GLEN
OA 712-33
OXFORD
TIBOR
QO 565.7
QO 220.9
DORVAL
QO 565.14
LASALLE
CAPITAL
QO 576.11
LAMAR
QO 574.21
YAMASKA
MARION
BALDWIN
MANIC
KAMOURASKA
LAURENT
SHAW
QO 576.39
QO 573.10
QO 576.27
QO 573.5
NOVA
DONALD
NZ-3
CLUSTER #1
CLUSTER #2
CLUSTER #3
CLUSTER #4
CLUSTER #5
CLUSTER #6

loading PC_1 with eigenvalues ranging from 0.38 to 0.42. The PC_1 vs. PC_2 plot (Fig. 2b) shows the large influence of the first principal component in separating the oat genotypes and the orientation of the 5 superimposed clusters from the CA. The dendrogram from the CA shows the 33 genotypes grouped into 5 distinct clusters with an R^2 value of 87% (Fig. 2c). Of the 8 oat genotypes located in the first cluster 7 of them denoted high levels of partial resistance to *P. coronata* race 264 were OA 712-17, Woodstock, QO 220.13 and Glen. The cultivar Newman which was part of the first cluster is completely resistant to race 264 but had low levels of crown rust infection. The latter was probably due to a small influx of natural inoculum of *P. coronata* which was virulent on this cultivar. The last cluster contained 2 genotypes with very low levels of partial resistance, they were Manic and the susceptible check NZ-3.

Similarly, the principal components PC_1 and PC_2 from the PCA of the 1990 field trial explained 85% and 8% respectively, of the variation attributed to the CPLADT variables on each of the five sampling dates. The component loading of PC_1 by the CPLADT values was very similar for all sampling dates, with eigenvalues ranging from 0.41 to 0.47. The PC_1 v. PC_2 plot results were showed the oat genotypes being separated mostly by PC_1 and with a total of 6 superimposed clusters (Fig. 3b). The latter results are comparable to those obtained for the 1989 trial in terms of the location of the genotypes in the lefthand side of the PC plot. The dendrogram from the CA from the 1990 field trial based on the CPLADT values identified 6 clusters with a R^2 of 83%. (Fig. 3c). The first

cluster had 7 genotypes which were Newman, QO 224.5, QO 220.13, Fidler, Woodstock, OA 712-17 and Glen. The second cluster had only one constituent which was OA 712-33, this breeding line was also located in the top of the second cluster in the dendrogram of the 1989 field trial. The last cluster in the dendrogram consisted solely of the susceptible check NZ-3.

Field Study Based on Flag Leaf Only

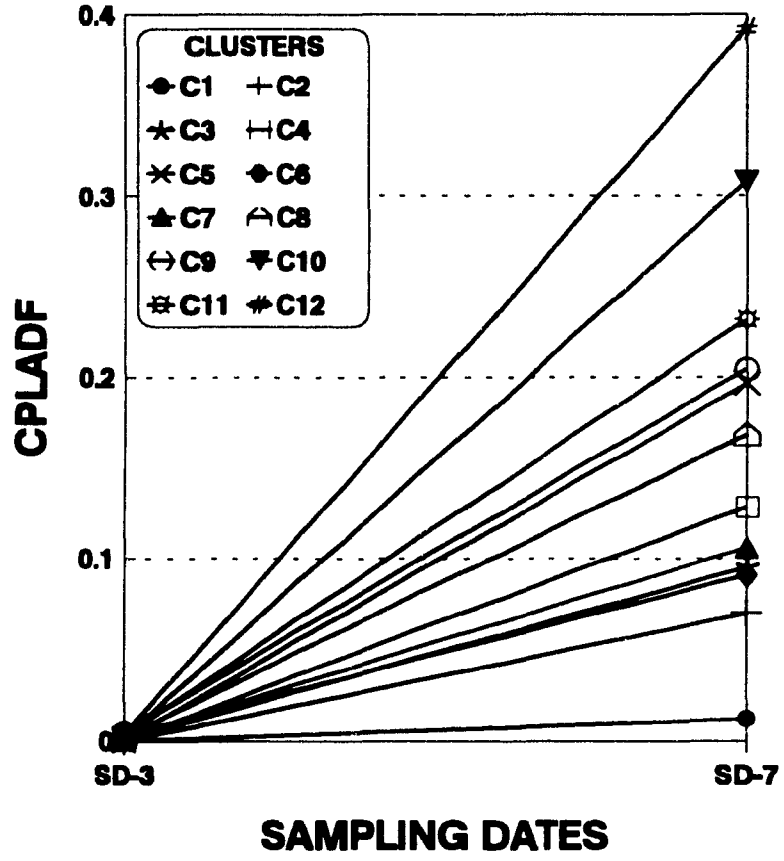
The disease progress curves for the CPLADF values averaged for each cluster of oat genotypes for both trials are shown in Figures 4a and 5a respectively. The latter figures denote a delayed disease progress for all oat genotypes as compared to the CPLADT results, because the flag leaf is the last leaf to appear, but the general trend of the disease progress curves for each oat genotype is comparable to those CPLADT values.

Devising a Simplified Field Sampling Method for Crown Rust of Oat

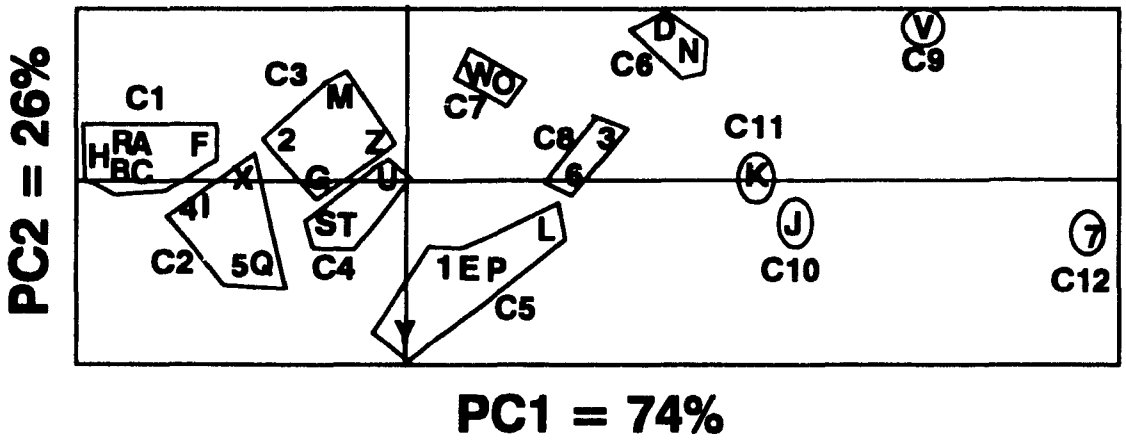
All combinations of CPLADF values at different sampling dates for all 33 oat genotypes for both field trials separately, were submitted to the PCA and CA in order to obtain a combination that would use the at least two or more sampling dates and still give results comparable to the previous PCA and CA with the CPLADT values over all

Fig 4: A. Disease progress of *P. coronata* race 264, expressed as the cumulative proportion of leaf area diseased (CPLADF) based on the flag leaf sampled for oat genotypes within each of 12 clusters during the 1989 field trial. B. The plot of PC_1 v. PC_2 showing the location of each oat genotype in respect to each other and the results of the cluster analysis are superimposed onto the plot equally showing the distances between the cluster groupings relative to each other. C. The dendrogram of the CPLADT values for the sampling dates SD-3 and SD-7 for each oat genotype (R^2=0.96).

4A


CLUSTERS
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
C11
C12
0.4
0.3
0.2
0.1
0
CPLADF
SD-3
SD-7
SAMPLING DATES

4B


PC2 = 26%
C1
C3
C2
C4
C5
C6
C7
C8
C9
C10
C11
C12
PC1 = 74%

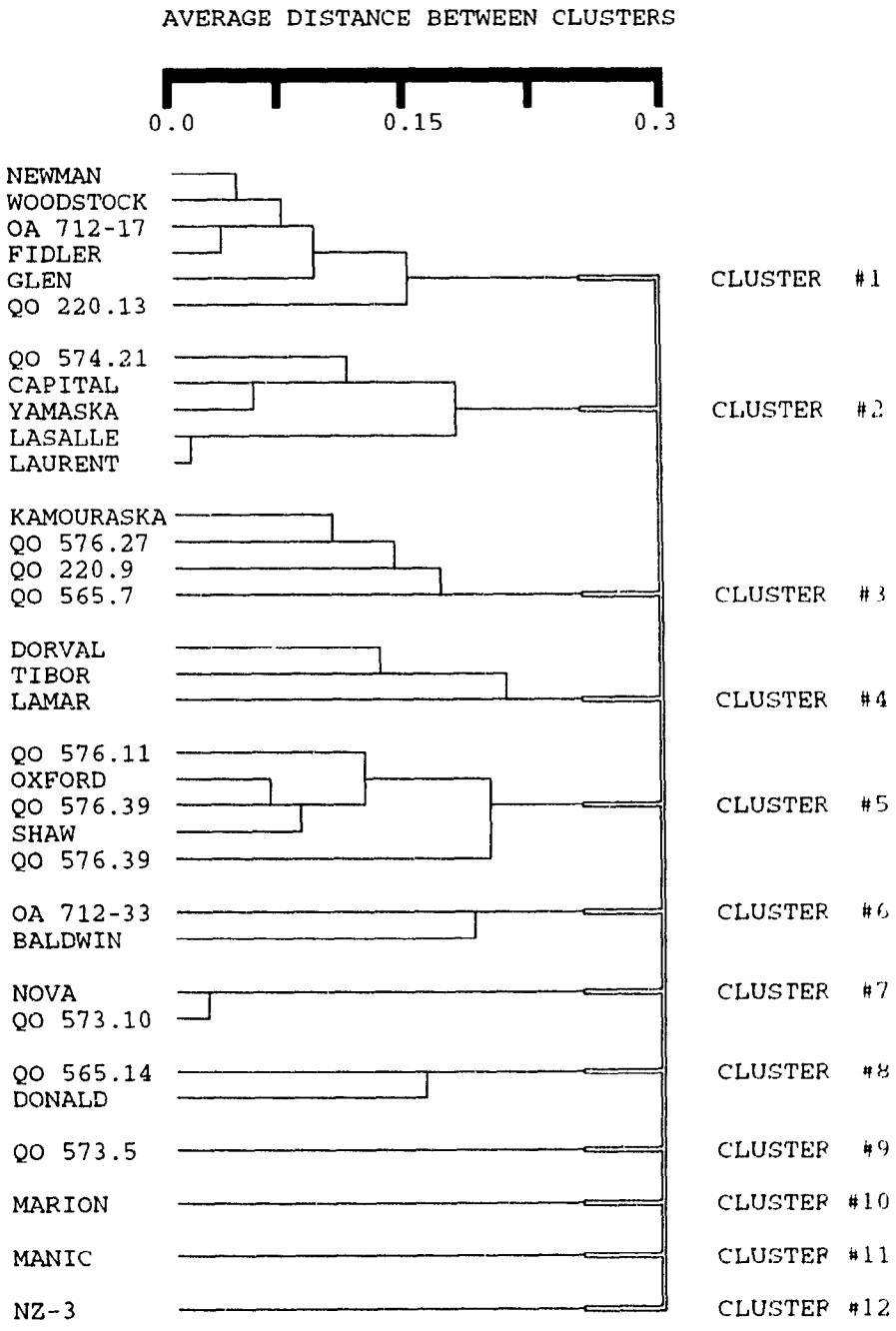
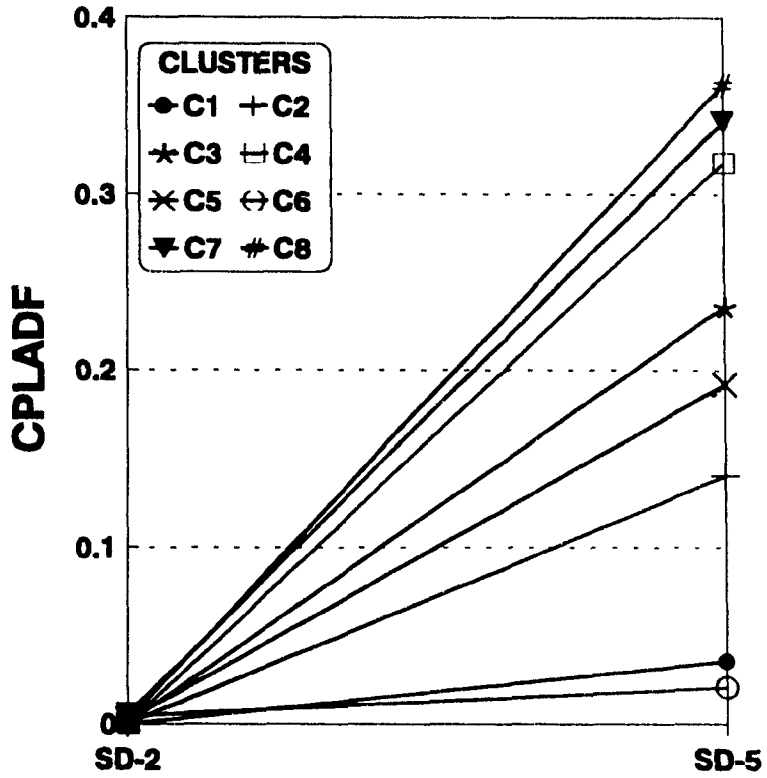
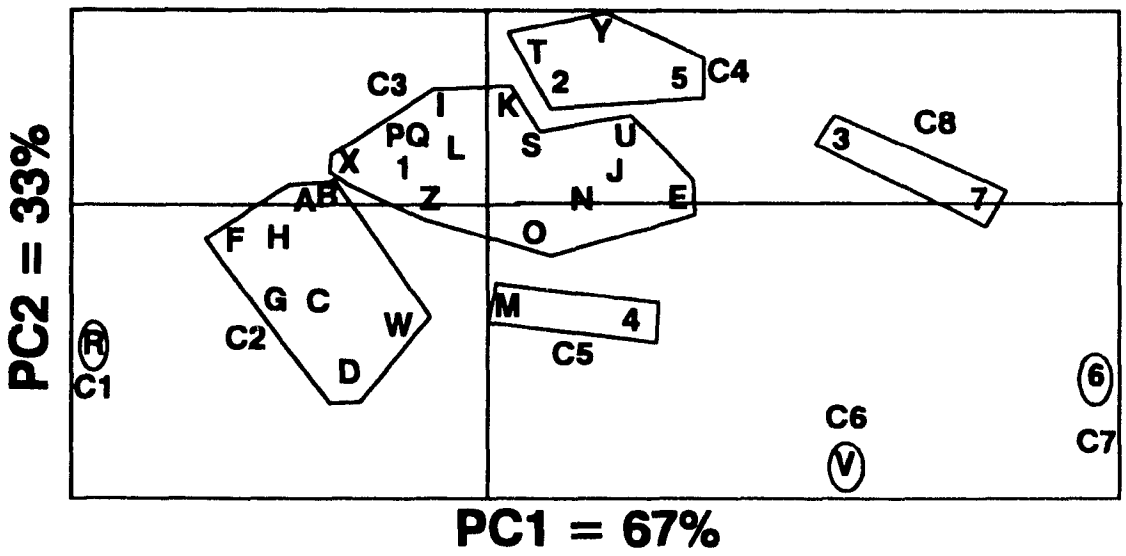

AVERAGE DISTANCE BETWEEN CLUSTERS
0.0
0.15
0.3
NEWMAN
WOODSTOCK
OA 712-17
FIDLER
GLEN
QO 220.13
CLUSTER #1
QO 574.21
CAPITAL
YAMASKA
LASALLE
LAURENT
CLUSTER #2
KAMOURASKA
QO 576.27
QO 220.9
QO 565.7
CLUSTER #3
DORVAL
TIBOR
LAMAR
CLUSTER #4
QO 576.11
OXFORD
QO 576.39
SHAW
QO 576.39
CLUSTER #5
OA 712-33
BALDWIN
CLUSTER #6
NOVA
QO 573.10
CLUSTER #7
QO 565.14
DONALD
CLUSTER #8
QO 573.5
CLUSTER #9
MARION
CLUSTER #10
MANIC
CLUSTER #11
NZ-3
CLUSTER #12

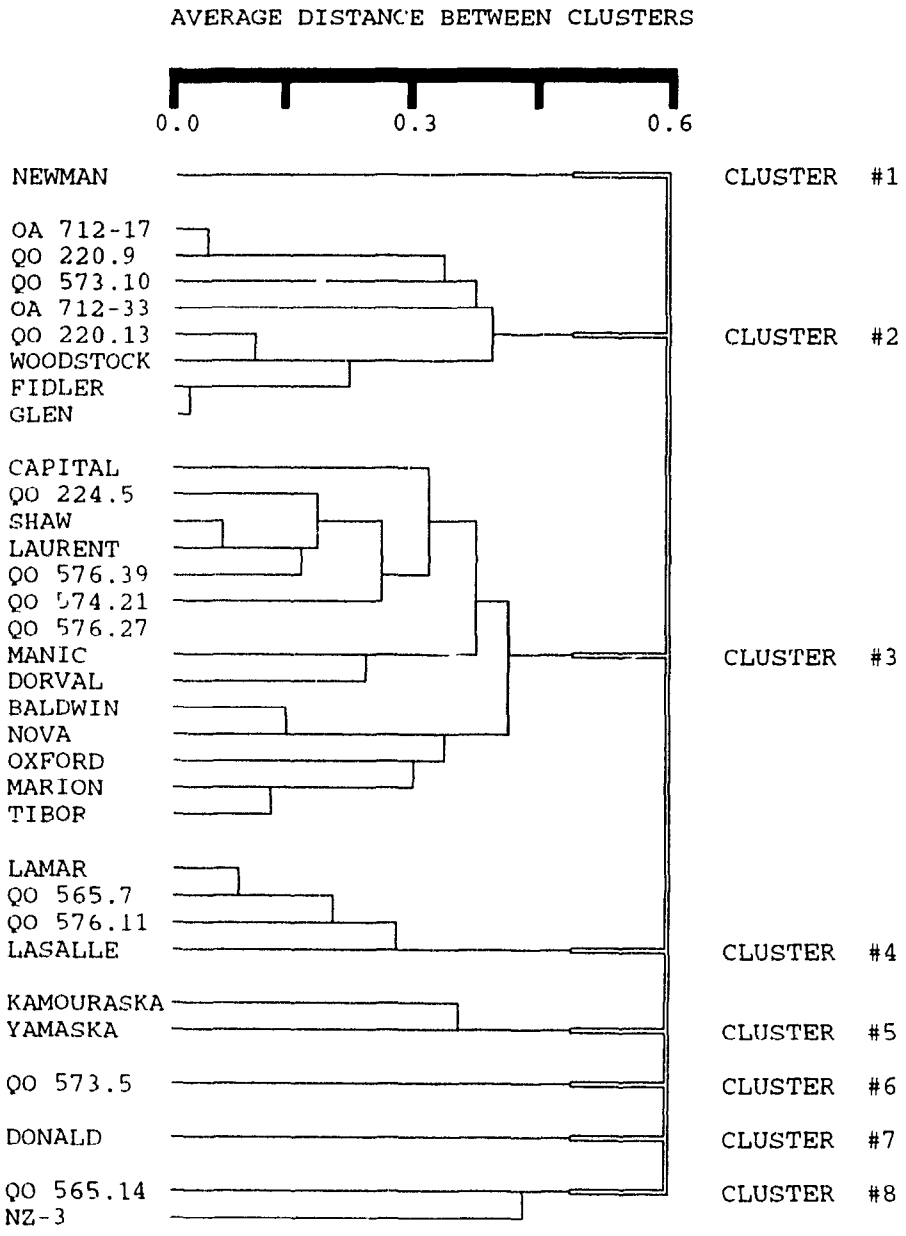
Fig 5: A. Disease progress of *P. coronata* race 264, expressed as the cumulative proportion of leaf area diseased (CPLADF) based on the flag leaf sampled for oat genotypes within each of 8 clusters during the 1990 field trial. B. The plot of PC_1 v. PC_2 showing the location of each oat genotype in respect to each other and the results of the cluster analysis are superimposed onto the plot equally showing the distances between the cluster groupings relative to each other. C. The dendrogram of the CPLADT values for the sampling dates SD-2 and SD-5 for each oat genotype (R^2=0.82).

5A


CLUSTERS
C1
C2
C3
C4
C5
C6
C7
C8
0.4
0.3
0.2
0.1
0
CPLADF
SD-2
SD-5
SAMPLING DATES

5B


PC2 = 33%
PC1 = 67%
C1
C2
C3
C4
C5
C6
C7
C8
R
V
6
T
Y
2
5
3
7
I
K
PQ
L
S
U
X
1
J
AB
Z
N
E
O
F
H
G
C
W
D
M
4


AVERAGE DISTANCE BETWEEN CLUSTERS
0.0
0.3
0.6
NEWMAN
OA 712-17
QO 220.9
QO 573.10
OA 712-33
QO 220.13
WOODSTOCK
FIDLER
GLEN
CAPITAL
QO 224.5
SHAW
LAURENT
QO 576.39
QO 574.21
QO 576.27
MANIC
DORVAL
BALDWIN
NOVA
OXFORD
MARION
TIBOR
LAMAR
QO 565.7
QO 576.11
LASALLE
KAMOURASKA
YAMASKA
QO 573.5
DONALD
QO 565.14
NZ-3
CLUSTER #1
CLUSTER #2
CLUSTER #3
CLUSTER #4
CLUSTER #5
CLUSTER #6
CLUSTER #7
CLUSTER #8

sampling dates for both field trials. This trial and error method yielded a combination that was similar to the previous results but used only 2 sampling dates, SD-3 and SD-7 (1989) and SD-2 and SD-5 (1990). These sampling dates corresponded to the averaged Zadoks growth stages of 52 and 85 (Table 2).

Principal Component and Cluster Analyses of the Field Studies Based on the Flag Leaf Only

The results from the PCA and CA for both trials of the field experiment using the CPLADF values for only two sampling dates are shown in Figures 4 and 5 respectively. The principal components PC_1 and PC_2 from the PCA of the 1989 field trial, explained 74% and 26% of the original variation respectively attributed to the CPLADF variables on each of the six sampling dates. The CPLADF values for both sampling dates contributed equally in loading PC_1 with eigenvalues of 0.71 for both sampling dates. The PC_1 v. PC_2 plot (Fig. 4b) shows the large influence of the first principal component in separating the 33 oat cultivars and breeding lines and the orientation of the 12 superimposed clusters from the CA. The dendrogram of the CA for the 1989 CPLADF values for sampling dates SD-3 and SD-7 gave 12 clusters with a R^2 of 96% (Fig. 4c). The 6 genotypes in the first cluster are Newman, Woodstock, OA 712-17, Fidler, Glen and QO 220.13. The last cluster had only one member which was the susceptible check NZ-3.

The principal components for the 1990 field trial using the CPLADF values for sampling dates SD-2 and SD-5 had values of 67% and 33% respectively for PC_1 and PC_2. The eigenvalues for sampling dates SD-2 and SD-5 loaded the first principal component equally with a value of 0.71 for both sampling dates. The PC_1 vs. PC_2 plot shows the large separation of the 8 superimposed clusters due to the large PC_1 value (Fig. 5b). The dendrogram for the 1990 CPLADF values for sampling dates SD-2 and SD-5 gave 8 clusters with a R^2 of 82% (Fig. 5c). The first cluster had only one constituent which was the cultivar Newman, the second cluster had 8 genotypes which were Woodstock, Fidler, QO 220.13, Glen, OA 712-17, QO 220.9, QO 573.10 and OA 712-33. The last cluster in this dendrogram was comprised of the oat lines QO 565.14 and NZ-3.

Controlled Environment Study

The results for both trials of the growth bench study are summarized in Tables 3 and 4 respectively. The results for the MLP in days ranged 2.8 days and 1.7 days between the highest and lowest values respectively for both trials. The results for MAXLES showed there were differences of 133 lesions and 72 lesions respectively, for both trials between the highest and lowest values. The LSAREA parameter measured for each oat genotype had differences between the largest and smallest lesion areas of 0.60mm^2 and 0.45mm^2 for both trials respectively.

Table 3. The averaged values for mean latency period (MLP), maximum number of lesions (MAXLES), and lesion area (LESAREA) for each of the 32 oat genotypes in the first growth bench experiment.

OAT GENOTYPE	MLP (DAYS)	MAXLES	LSAREA (mm^2)
GLEN	12.5	59	0.58
FIDLER	12.8	44	0.41
OA 712-17	13.1	16	0.38
OA 712-33	12.3	38	0.28
OXFORD	11.7	42	0.58
QO 220.13	12.2	67	0.48
QO 220.9	11.6	105	0.82
WOODSTOCK	12.7	34	0.43
CAPITAL	12.1	97	0.73
MARION	11.7	111	0.69
MANIC	10.3	149	0.77
QO 224.5	11.2	102	0.45
KAMOURASKA	11.3	62	0.56
BALDWIN	11.5	82	0.66
NOVA	11.4	54	0.51
SHAW	11.8	53	0.74
LAURENT	11.2	98	0.63
DORVAL	11.5	58	0.54
LAMAR	10.9	75	0.61
TIBOR	12.1	62	0.48
QO 573.5	11.6	76	0.63
QO 573.10	10.7	118	0.62
QO 574.21	11.9	72	0.59
QO 576.11	11.2	121	0.71
QO 576.27	11.5	87	0.47
QO 576.39	11.7	61	0.53
QO 565.7	11.6	80	0.61
QO 565.14	11.3	91	0.65
YAMASKA	11.7	90	0.73
LASALLE	11.3	46	0.65
DONALD	11.4	49	0.66
NZ-3	11.3	100	0.88

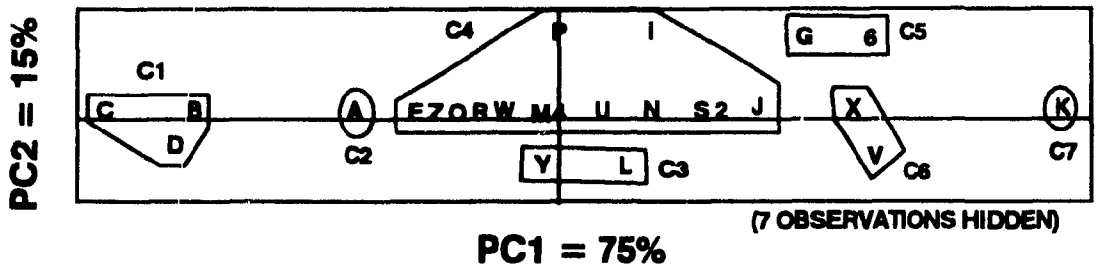
Table 4. The averaged values for mean latency period (MLP), maximum number of lesions (MAXLES), and lesion area (LESAREA) for each of the 32 oat genotypes in the second growth bench experiment.

OAT GENOTYPE	MLP (DAYS)	MAXLES	LSAREA (mm^2)
GLEN	12.1	81	0.54
FIDLER	12.3	53	0.49
OA 712-17	13.1	35	0.36
OA 712-33	12.9	53	0.42
OXFORD	11.4	85	0.51
QO 220.13	12.3	64	0.42
QO 220.9	11.9	61	0.64
WOODSTOCK	12.4	28	0.53
CAPITAL	11.8	55	0.69
MARION	12.1	100	0.52
MANIC	11.3	49	0.67
QO 224.5	12.1	66	0.42
KAMOURASKA	12.2	90	0.65
BALDWIN	11.7	93	0.51
NOVA	11.6	48	0.61
SHAW	12.1	50	0.59
LAURENT	11.9	94	0.47
DORVAL	11.9	45	0.57
LAMAR	12.1	58	0.54
TIBOR	11.9	66	0.53
QO 573.5	11.3	85	0.49
QO 573.10	11.8	92	0.52
QO 574.21	12.4	89	0.63
QO 576.11	12.1	63	0.61
QO 576.27	11.8	71	0.56
QO 576.39	12.3	57	0.56
QO 565.7	12.1	57	0.56
QO 565.14	11.8	85	0.55
YAMASKA	12.1	83	0.61
LASALLE	12.1	88	0.57
DONALD	11.4	82	0.62
NZ-3	11.7	86	0.81

Principal Component and Cluster Analyses of the Controlled Environment Study

The first two principal components for the first trial explained 75% and 15% of the variability respectively for PC_1 and PC_2. The eigenvalues for PC_1 were 0.58, 0.60 and 0.55 respectively for MLP, MAXLES and LSAREA. The plot of PC_1 vs PC_2 (Fig 6a) showed the influence of PC_1 in separating the oat genotypes from each other and had 7 cluster superpositioned onto it. The dendrogram of the CA for the first trial gave 7 clusters with a R^2 of 71% (Fig. 7). The three genotypes in the first cluster of the dendrogram are OA 712-17, OA 712-33 and Woodstock and the second cluster had the breeding lines QO 220.13 and QO 224.5. The last cluster had only one constituent, which was the breeding line NZ-3. The principal components for the second trial of the growth bench experiment explained 60% and 33% of the variability respectively for PC_1 and PC_2. The eigenvalues for PC_1 were 0.68, 0.45 and 0.57 respectively for MLP, MAXLES and LSAREA. The plot of PC_1 vs. PC_2 shows the influence of both principal components in separating the oat genotypes and the 7 superpositioned clusters (Fig 6b) The dendrogram for the second trial gave 7 clusters with a R^2 of 78% (Fig 8) The four genotypes in the first cluster are OA 712-17, OA 712-33, Woodstock and Fidler and the second cluster had Glen, QO 220.13 and Tibor. The last cluster had the three oat genotypes QO 576.11, QO 573.10 and Manic.

6A



6B

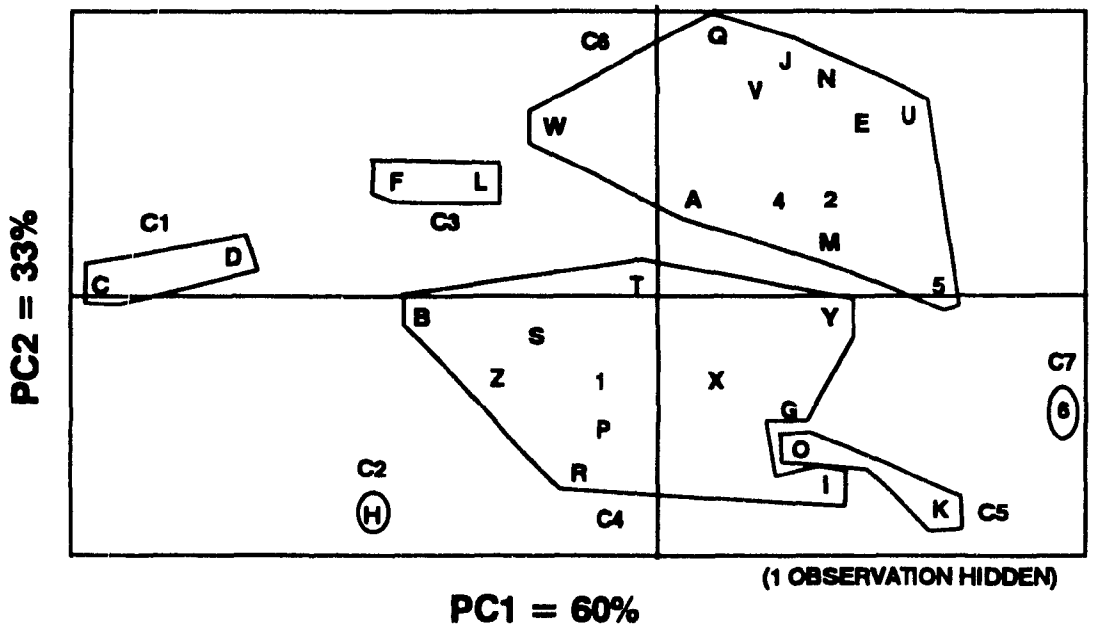


Fig 6. The clusters of the 32 oat genotypes superimposed on to the plots of the first two principal components from the first trial (6A) and second trial (6B) of the growth bench experiment.

Fig 7. The dendrogram of the cluster analysis using MLP, MAXLES and LSAREA disease parameters for each oat cultivar and breeding line (R^2=0.71)

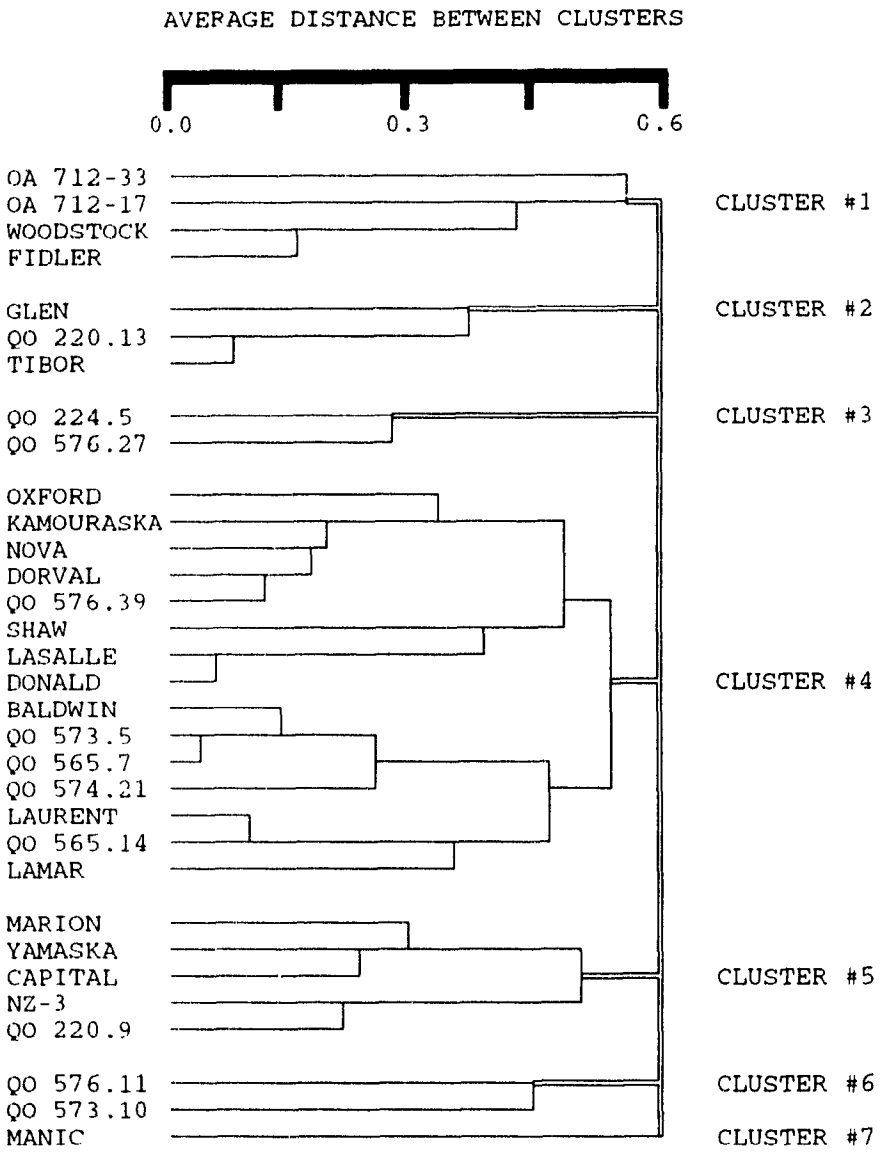
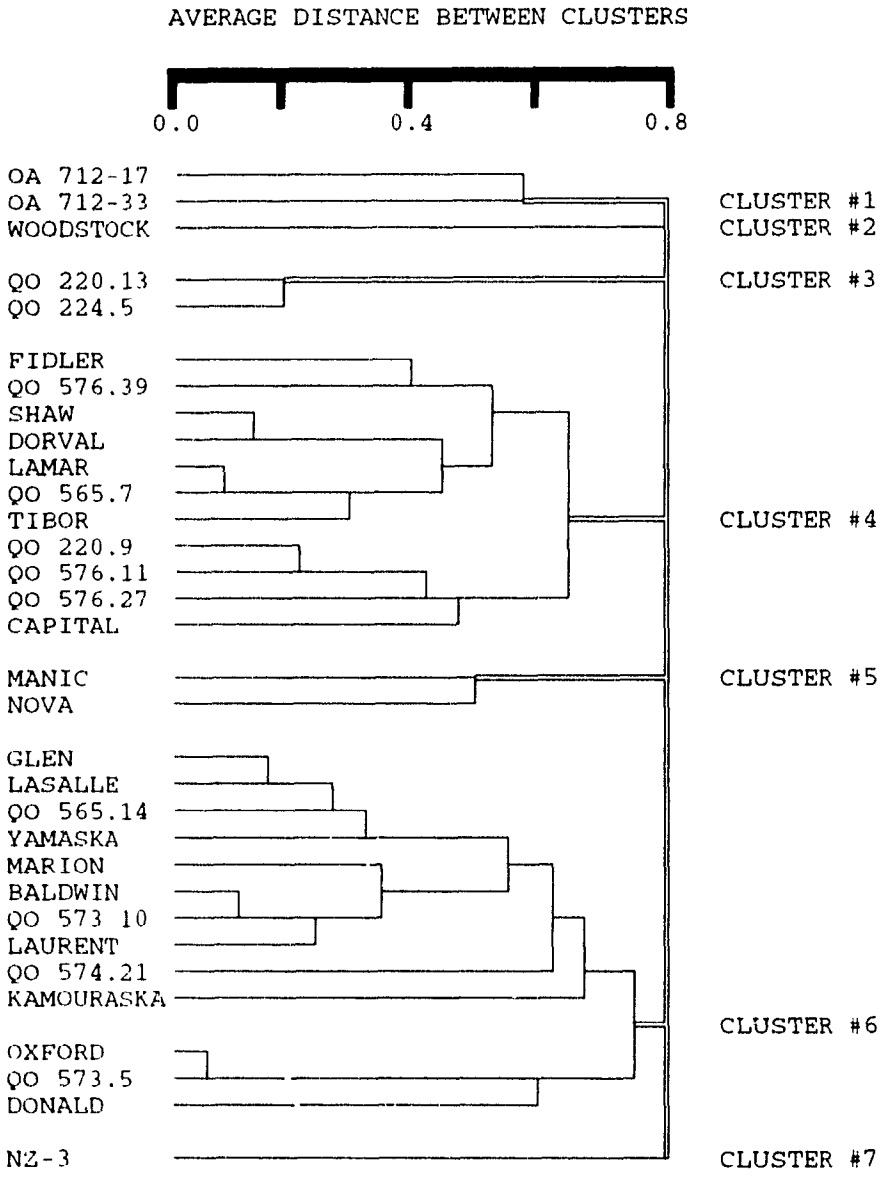

AVERAGE DISTANCE BETWEEN CLUSTERS
0.0
0.3
0.6
OA 712-33
OA 712-17
WOODSTOCK
FIDLER
GLEN
QO 220.13
TIBOR
QO 224.5
QO 576.27
OXFORD
KAMOURASKA
NOVA
DORVAL
QO 576.39
SHAW
LASALLE
DONALD
BALDWIN
QO 573.5
QO 565.7
QO 574.21
LAURENT
QO 565.14
LAMAR
MARION
YAMASKA
CAPITAL
NZ-3
QO 220.9
QO 576.11
QO 573.10
MANIC
CLUSTER #1
CLUSTER #2
CLUSTER #3
CLUSTER #4
CLUSTER #5
CLUSTER #6
CLUSTER #7

Fig 8. The dendrogram of the cluster analysis using MLP, MAXLES and LSAREA disease parameters for each oat cultivar and breeding line (R^2=0.78).


AVERAGE DISTANCE BETWEEN CLUSTERS
0.0
0.4
0.8
OA 712-17
OA 712-33
WOODSTOCK
QO 220.13
QO 224.5
FIDLER
QO 576.39
SHAW
DORVAL
LAMAR
QO 565.7
TIBOR
QO 220.9
QO 576.11
QO 576.27
CAPITAL
MANIC
NOVA
GLEN
LASALLE
QO 565.14
YAMASKA
MARION
BALDWIN
QO 573 10
LAURENT
QO 574.21
KAMOURASKA
OXFORD
QO 573.5
DONALD
NZ-3
CLUSTER #1
CLUSTER #2
CLUSTER #3
CLUSTER #4
CLUSTER #5
CLUSTER #6
CLUSTER #7

DISCUSSION

The PCA and CA analyses of disease parameters such as CPLADT, CPLADF, MLP, MAXLES, and LSAREA proved to be very useful in grouping the 33 oat cultivars and breeding lines into distinct groups with similar resistance characteristics to *P coronata* race 264.

The oat cultivars and breeding lines in the first cluster of the 1989 (CPLADT) and first two clusters of 1990 (CPLADT) field trials had a very small slopes of disease progress as well as lower final disease levels as compared to the other clusters plotted. If each individual genotype was plotted separately and evaluated as such we you have a difficult time selecting a group of highly resistant genotypes using mean comparison statistical procedures as compared to CA and PCA analyses.

By using various combinations of disease severity (CPLADF) on different sampling dates in the PCA and CA, it was possible to determine a simplified sampling method that required only 2 sampling occasions to evaluate partial resistance to crown rust in a group of oat genotypes. Though more clusters were identified, the cultivars and breeding lines Newman, OA 712-12, QO 220.13, Woodstock and OA 712-33 appeared in the first two clusters in both years. There also tended to be more movement of some oat cultivars and breeding lines between clusters, this was attributed to the fact there were only two sampling dates used and that the cluster analysis gave equal weight to both of

them. Some oat genotypes with high final disease levels had no disease present on SD-2 (1989) or SD-3 (1990), as compared to some genotypes with low final disease levels and had some disease present on the latter sampling dates. This tended to place the former type of genotype in a cluster with genotypes of higher partial resistance and the opposite is true for the latter types of genotypes. Therefore to correct for this effect more weight could be given to the CPLADF value on the last sampling date, but how much more weight to assign is a difficult question.

The individual maturity of the cultivars and breeding lines under field conditions and its effects on the resistance to *P. coronata* was not specifically addressed. It is expected that early maturing genotypes would have a reduced rate of epidemic buildup of *P. coronata* during the growing season. The oat genotypes under study were relatively even in their rate of maturity with the exception of QO 220.13 which was slightly later maturing and Glen which was slightly earlier maturing. However these differences only varied several days each way and were not important enough to substantially alter the clusters of oat genotypes, because all the genotypes were sampled until grain filling had ceased.

The clusters displaying high levels of partial resistance selected from the growth bench study were similar to those obtained in the field study, as they contained many of the same oat genotypes. There was more variation in the ranking of oat genotypes from the middle of the dendrograms on downwards The cultivars and breeding lines with high

levels of partial resistance always ranked in the top two clusters in all dendrograms. The cultivar Glen was an exception to this as it showed up in the 6th cluster in the first growth bench trial, this was mainly due to its sparse growth habit in the field which was not expressed in the growth bench experiment. Glen's thin leaves when grown under field conditions would keep this cultivar's canopy humidity level much lower than most other cultivars with bushy growth habits. This growth bench study offered a quick method (30 days from sowing to the appearance of the last uredia) to screen oat genotypes for partial resistance, but this remains labour intensive to conduct.

The cultivar Newman though resistant to race 264, showed some crown rust symptoms in the field during both years. This was due to natural inoculum that was present in the field even though the field inoculation with race 264 was done early enough so that this race would be the most prevalent race during the course of the experiment. However if required, further field screening could be done with mixtures of races, especially the more aggressive ones. The use of a mixture of races would be more representative of natural field conditions.

In conclusion the following oat cultivars and breeding lines were found to have high levels of partial resistance to *P. coronata* race 264: OA 712-17, OA 712-33, Glen, Woodstock, QO 220.13 and QO 574.21. The simplified field screening method proposed here requires quantification of disease on the flag leaf at the Zadoks growth stages 53 and 85. Despite the fact that Newman showed disease in the field, it denoted a high level of

partial resistance to naturally occurring populations of *P. coronata*. In future studies the quantification of leaf area should be considered as a possible resistance parameter for screening for partial resistance to *P. coronata*.

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CONNECTING TEXT

The selection of partial resistance of oat cultivars and breeding lines under both controlled environmental and field conditions has been accomplished in the previous study. The disease parameters that were used successfully to select for partial resistance to *Puccinia coronata* race 264 were, mean latency period, maximum number of lesions, and mean lesion area (under growth bench conditions) and the cumulative proportion of leaf area diseased (under field conditions). Sporulation has been shown to be a good selection parameter for partial resistance in wheat and barley rust diseases (Mehta and Zadoks, 1970; Neervoort and Parlevliet, 1978). This prompted a further investigation to study the sporulation characteristics of seven oat genotypes selected from the previous investigation with known levels of partial resistance and to evaluate the suitability of this parameter as a potential selection parameter for partial resistance to *P. coronata* race 264 under controlled environmental conditions. The same seven oat cultivars and breeding lines were also studied further under field conditions with large plots to verify the results obtained in the growth bench study and to compare with the previous field study to determine if any interplot interference may have occurred.

References

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III. PARTIAL RESISTANCE TO CROWN RUST (*Puccinia coronata* Cda.) RACE *264* IN SEVEN OAT CULTIVARS AND BREEDING LINES

INTRODUCTION

Crown rust of oat caused by *Puccinia coronata* Cda. is a foliar pathogen of worldwide importance (Fig. 1a) and is quite severe in places where its alternate host *Rhamnus cathica* L. is present. Complete resistance to all races of the pathogen is the only feasible method of controlling this disease but this increases the selection pressure on the pathogen resulting in the development of new virulent races. This "boom and bust" cycle of rust resistance eventually occurs with all newly released cultivars including the eastern Canadian cultivar Woodstock which lost its vertical resistance to all prevalent races of *P. coronata* within three years after its release (Chong,1988).

An alternative to complete major gene (vertical) resistance is the use of partial (horizontal) resistance. Partial resistance is characterized by a reduced rate of epidemic build-up despite a high, susceptible infection type, by absence of large race specific effects and durability (Parlevliet, 1988). The breeding of cultivars with high levels of partial resistance extend the useful life of new cultivars by decreasing the selection pressure on *P. coronata* to develop new races.

Quantitative disease resistance parameters have been shown to be quite valuable for selecting for partial resistance under both field and controlled environmental conditions with the exception of yield parameters which have been proven to be unreliable (Politowski, 1978; Singleton, 1982). Application of multivariate analyses such as principal component and cluster analyses have proven valuable for the selection of useful disease parameters and the grouping of plant genotypes of similar resistance characteristics (Jeger, 1980; Lebeda and Jendrulek, 1987, Anderson, 1990).

The objectives of this investigation were to study the sporulation characteristics in seven oat cultivars and breeding lines with different levels of partial resistance selected from a previous investigation, and to evaluate the suitability of this parameter as well as the mean latency period and total lesions/leaf as potential mass screening parameters for partial resistance in oat to *P. coronata* under controlled environmental conditions. The same oat genotypes were also studied further under field conditions to compare with the controlled environmental study.

MATERIALS AND METHODS

Controlled Environment Study

Three oat cultivars and four breeding lines (Table 5) with different levels of partial

Table 5. List of oat cultivars and breeding lines used in the field and growth bench experiments, and their level of partial resistance to *Puccinia coronata* race 264.

OAT GENOTYPE	LEVEL OF RESISTANCE	IDENTIFICATION CHARACTER *
GLEN	HIGH PARTIAL RESISTANCE	A
OA 712-17	HIGH PARTIAL RESISTANCE	B
QO 220.13	HIGH PARTIAL RESISTANCE	C
WOODSTOCK	HIGH PARTIAL RESISTANCE	D
QO 574.21	HIGH PARTIAL RESISTANCE	E
YAMASKA	MEDIOCRE PARTIAL RESISTANCE	F
NZ-3	LOW PARTIAL RESISTANCE	G

*** Note: The identification character is used to identify each genotype in the principal component plots.**

resistance selected from a previous study (See chapter II) were sown in 10cm pots at the rate of 4 seeds/pot and placed on a growth bench set at 20°C in a randomized complete block design with 4 replicates. After the second leaf had fully expanded, each pot was thinned to one plant/pot and the second leaf of each plant was held in a horizontal position with a plastic strip and a paper clip to maximize inoculum deposition during inoculation. Two complete replicates at a time were inoculated in an automated spray chamber (custom built by Research Instrument Mfg. Co. Ltd.) by spraying a suspension of freshly collected uredospores of *P. coronata* race 264 mixed in Isopar M© oil adjusted at 200 000 spores/ml. The nozzle speed was adjusted at 2.4 km/hr, the pressure to 175 kPa and the height to 61cm (Fig. 1b). The inoculated plants were incubated in a dew chamber at 20°C for a period of 48hrs and returned to a growth bench maintained at 20°C. The second leaf was trimmed to a length of 15cm in order to fit in the 15cm test tubes. These leaves were then inserted into 15cm clear polyethylene test tubes which were ventilated with small holes 2cm apart and a 3cm slit on the top surface to avoid condensation inside the tubes. The inside of the tubes had been previously washed with Radio Shack© Antistatic spray to eliminate static and the bottom was lined with a wax paper to facilitate the deposition and removal of spores. The test tubes were held in place with clamps attached to an aluminum rod which was suspended with stands (Fig. 1c,d).

On the seventh day after inoculation the number of sporulating uredia were counted daily on the top and bottom surfaces of each inoculated leaf of each pot until all sporulating uredia had appeared (on day 16) and the mean latency period (MLP) was

calculated for each leaf by using the equation (Eq. 2) developed by Shaner (1978). After all the pustules had erupted on both the top and bottom leaf surfaces, the total number of rust pustules was calculated (MAXLES) by summing the totals for both leaf surfaces.

[2]

$$\mathbf{MLP} = \sum \mathbf{p_i}\, \mathbf{T_i}$$

Where MLP is mean latency period

p_i = the proportion of maximum pustules formed on the i^{th} day after inoculation.

T_i = the total number of days since inoculation until the i^{th} day.

Commencing from the 10^{th} day after inoculation and every 3 days thereafter, spores were collected from each tube by first tapping on the leaf and subsequently carefully withdrawing it out of the tube. Then the spores were poured out of the collecting tube into a screwcap test tube. The spore collecting tube was then replaced in the clamp and the leaf was reinserted into the tube. The spores were collected until sporulation had ceased in all leaves. Each spore sample was then suspended in a known volume of Isopar M© oil and counted four times using a hemacytometer and a mean number of spore/sample was calculated. The sporulation parameters calculated were the total

sporulation per leaf (TOTSPO) and the total sporulation/pustule (SPO/LES). The sporulation/pustule was calculated for each genotype by dividing the total sporulation for each oat genotype by their corresponding MAXLES values. The MLP, MAXLES, TOTSPO and SPO/LES for each genotype were used in the multivariate analyses to group oat genotypes with similar levels of partial resistance.

Field Study

The field study of this investigation was conducted once during the 1990 growing season at the Emile Lods Research Station of McGill University in Ste Anne de Bellevue, Québec. The three oat cultivars and four breeding lines (Table 5) used in the growth bench experiment were sown in 5.0 x 5.0m plots in a randomized complete block design with three replicates. Each plot consisted of 24 rows with 0.20m spacing and was seeded at the rate of 210 seeds/m and there was 5.0m spacing between each plot and 2.0m spacing between each block.

A hill of the oat line NZ-3 was seeded in the centre of each plot. Fifteen plants from each hill were syringe inoculated at the third leaf stage with 1mL of a suspension of freshly collected uredospores of *P. coronata* race 264 at approximately 100 000 uredospores/mL. Commencing at first sign of disease, five plants per plot were randomly selected and five leaves, the flag leaf and the 4 leaves preceding it, per plant were

nondestructively sampled,. Crown rust severity was visually estimated as the percent leaf area diseased (PLAD) at weekly intervals until the senescence of the flag leaf. The Distrain computer program (Tomerlin, 1988) was used as a training tool to visually estimate disease severity on oat leaves. The Zadoks growth stage for cereals (Zadoks, 1974) was assessed for each oat genotype on each sampling date.

The cumulative proportion of leaf area diseased (CPLAD) per plot for each sampling date was calculated using the DISPAR, a computer program that calculates various epidemiological disease parameters from disease severity data (Kushalappa and Carisse, 1989). The data on CPLAD for all five leaves sampled were used to group oat genotypes based on their rust resistance and to assess their partial resistance.

Data Analysis

Principal component analysis (PCA) was used in computing principal components to explain as much of the total variation among oat genotypes as possible with as few principal components as possible and to verify the weight of each variable on each principal component (Dillon and Goldstein, 1984). The results from the PCA is represented in two ways. The first two principal component proportions are shown in a pie chart and the weights of each eigenvector for the first principal component (PC_1) are represented as a stacked bar chart linked to the pie chart. This facilitates the display and comprehension of the results by graphically showing the size of each proportion and the

weight of each variable for the first principal component. The second representation of the PCA is by plotting PC_1 against PC_2 and by superimposing the results from the cluster analysis. The latter method has been used by Lebeda and Jendrulek (1988) and they pointed out that this method is as good as that of the minimum spanning tree to display clusters of similar plant genotypes. The minimum spanning tree was found to be superior to the dendrogram for the graphical presentation of similarities among varieties (Hau and Krantz, 1990). The PROC PRINCOMP procedure of the SAS© system was used to carry out the analysis (SAS, 1987) The variables of the growth bench study were standardized and the PCA was preformed on the correlation matrix with a variance of one because, the original values were not on the same scale of measurement.

Cluster analysis (CA) was used to group the oat cultivars and breeding lines based on the values of the rust resistance parameters quantified. The CA method used was the unweighed pair-group method using arithmetic averages which in the SAS© system this is equivalent to PROC CLUSTER - METHOD AVERAGE (SAS, 1987). The data best represents an analysis of objects (Q-analysis) and the data matrix was standardized in the growth bench study before analysis to remove the arbitrary effects due to the different scales of measurements of the variables. The CA results are shown graphically with the aid of a dendrogram (also known as a cluster tree) which displays the paired oat genotypes in clusters, the average distance between clusters and the coefficient of determination at the place where the tree was cut to form a classification.

RESULTS

Controlled Environment Study

The mean latency period (MLP) ranged from 11.9 to 14.9 days for the first trial and from 11.9 to 14.0 days for the second trial (Table 6). The oat genotypes that had the longest MLP were OA 712-17, QO 220.13 and Glen.

The total number of pustules/leaf (MAXLES) ranged from 92 to 504 lesion in the first trial and from 167 to 373 lesions for the second trial (Table 6). The oat genotypes that had the least number of pustules in both trial was OA 712-17.

The total number of spores (TOTSPO) produced by each genotypes ranged from 322 500 uredospores (OA 712-17) to 5 288 000 uredospores/leaf (Yamaska) for the first trial and from 510 000 uredospores (OA 712-17) to 3 357 000 uredospores/leaf (Yamaska) in the second trial (Table 6).

The sporulation/pustule (SPO/LES) ranged from 3525 (OA 712-17) to 11793 spores/pustule (QO 574.21) for the first trial and 3054 (OA 712-17) to 9000 (Yamaska) spores/pustule (Table 6). The data on MLP, MAXLES, TOTSPO and SPO/LES for each genotype and each trial were used as the variables in the PCA and CA analyses.

Table 6. The mean latency period (MLP), maximum number of lesions/leaf (MAXLES), total sporulation/leaf (TOTSPO), and total sporulation/lesion (TOTSPO/LES) for each of the seven oat genotypes in both trials of the growth bench study.

GROWTH BENCH: FIRST TRIAL

OAT GENOTYPE	MLP (DAYS)	MAXLES	TOTSPO	TOTSPO/LES
GLEN	12.7	331	2816500	8522
OA 712-17	14.9	92	322500	3525
QO 220.13	13.3	361	3488000	9662
WOODSTOCK	12.6	393	4714000	12003
QO 574.21	12.3	420	4950000	11793
YAMASKA	12.4	504	5288000	10502
NZ-3	11.9	415	4610500	11203

GROWTH BENCH: SECOND TRIAL

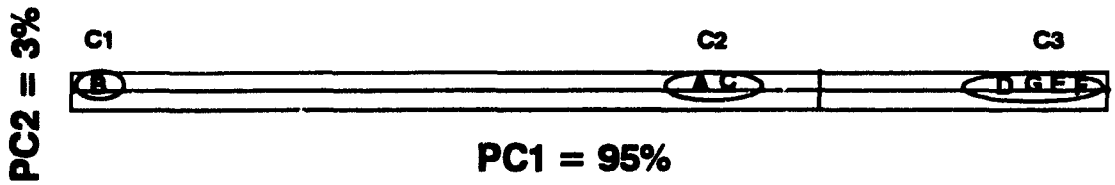
OAT GENOTYPE	MLP (DAYS)	MAXLES	TOTSPO	TOTSPO/LES
GLEN	12.8	230	1113000	4834
OA 712-17	14.1	167	510000	3054
QO 220.13	12.4	208	680500	3280
WOODSTOCK	12.8	276	2064500	7473
QO 574.21	12.6	356	2544667	7155
YAMASKA	11.9	373	3357000	9000
NZ-3	12.2	352	2850500	8104

Principal Component and Cluster Analyses of the Controlled Environmental Study

In the first trial, the first (PC_1) and second (PC_2) principal components explained 95% and 3% of the total variability in the original disease variables. Each of the original variables loaded PC_1 as follows: 0.51 for TOTSPO, 0.50 for MAXLES, 0.50 for SPO/LES and -0.49 for MLP. The plot of PC_1 vs. PC_2 shows the importance of PC_1 in separating the genotypes in the horizontal direction and also the separation of the superimposed clusters (Fig. 9a). The CA dendrogram for the first trial gave 3 cluster groupings; the first group contained only one breeding line (OA 712-17) with high partial resistance, the second group had 2 genotypes with high partial resistance (QO 220.13 and Glen) the last cluster grouping had 4 genotypes, including 2 with high partial resistance (Fig. 10a).

The results of the PCA for the second trial of the growth bench study were similar to the first trial. In the second trial PC_1 and PC_2 explained 88% and 10% of the variability in the original disease parameters respectively. The component loading of the PC_1 was; 0.52 for TOTSPO, 0.52 for MAXLES, 0.51 for SPO/LES and -0.44 for MLP. The large influence of PC_1 is seen in the plot of PC_1 vs. PC_2 where its influence in the horizontal direction helps to separate the oat genotypes and as well as the superimposed clusters in the horizontal direction (9b). The second trial gave 3 clusters; the first cluster had 3 genotypes with high partial resistance (OA 712-17, QO 220.13 and Glen), the second cluster had 1 cultivar (Woodstock) with high partial resistance and the third cluster had 3 oat genotypes, including one with high partial resistance (Fig. 10b).

9A



9B

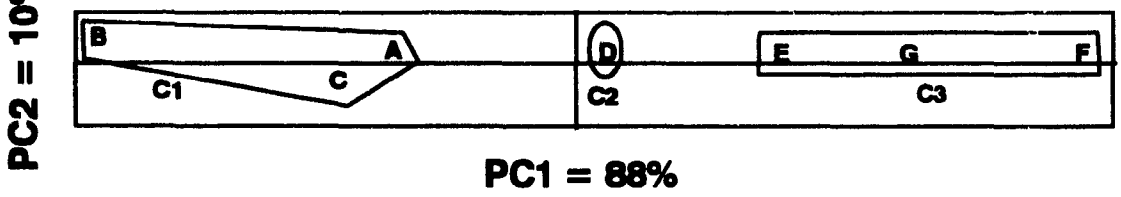
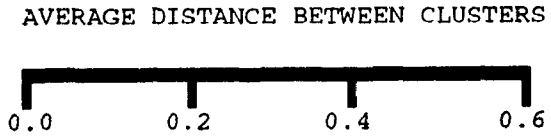
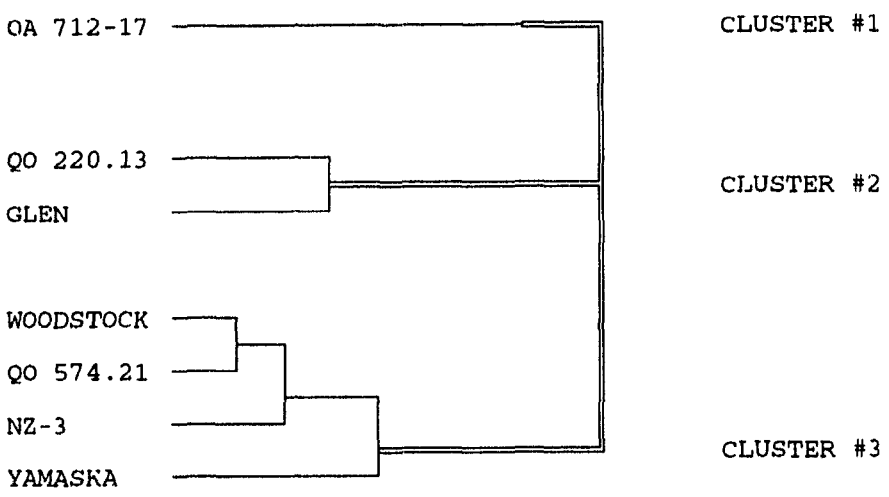


Fig 9. The superimposed clusters of the 7 oat genotypes on the plots of the first two principal components from the first trial (9A) and second trial (9B) of the growth bench experiment.

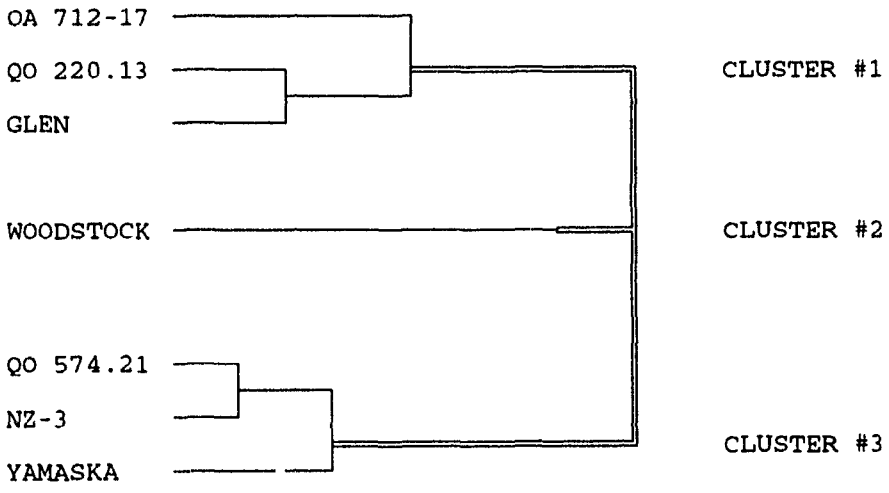
Fig. 10: A The dendrogram of the cluster analysis of the first growth bench trial using MLP, MAXLES, TOTSPO, and SPO/LES disease parameters for each oat genotype (R^2=0.80). B. The dendrogram of the cluster analysis of the second growth bench trial using MLP, MAXLES, TOTSPO, and SPO/LES disease parameters for each oat genotype (R^2=0.80).


AVERAGE DISTANCE BETWEEN CLUSTERS
0.0
0.2
0.4
0.6

10A


OA 712-17
QO 220.13
GLEN
WOODSTOCK
QO 574.21
NZ-3
YAMASKA
CLUSTER #1
CLUSTER #2
CLUSTER #3

10B


OA 712-17
QO 220.13
GLEN
WOODSTOCK
QO 574.21
NZ-3
YAMASKA
CLUSTER #1
CLUSTER #2
CLUSTER #3

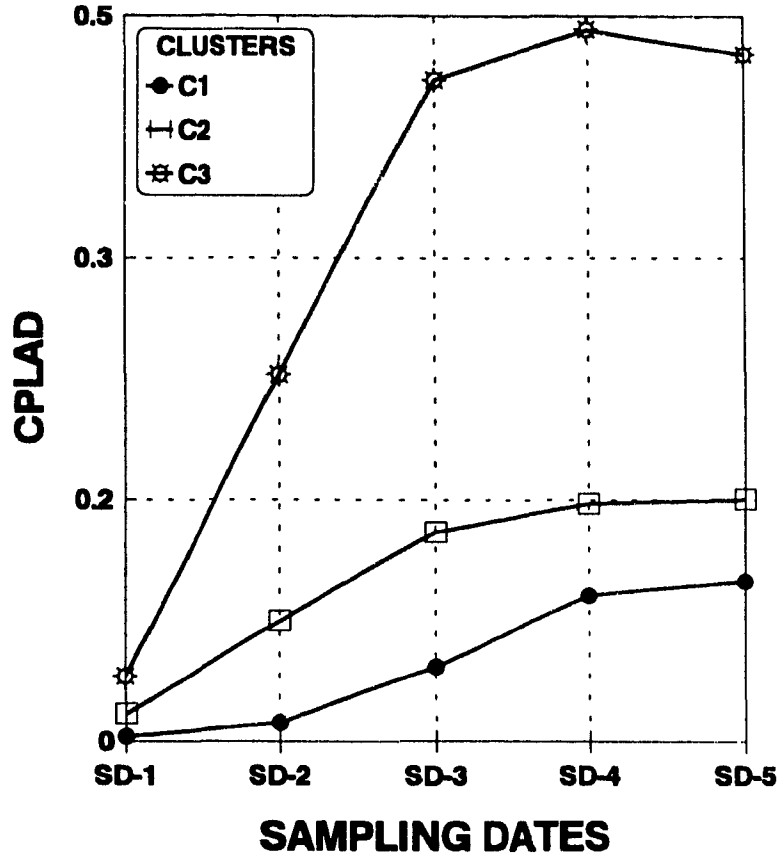
Field Study

During the course of the growing season each plot was sampled for a total of five times, and a mean disease progress curves for all oat genotypes within each cluster grouping is shown in Fig 11a. The first cluster's curve with a final CPLAD were below 0.18 contained all oat genotypes with high levels of partial resistance. The cultivar Yamaska performed better than expected and was grouped with the line QO 574.21 in the second cluster, this was due to the fact that QO 574.21 more closely resembled Yamaska than genotypes in the first cluster. The very susceptible NZ-3 in the third cluster by itself, had a large final CPLAD (0.43) and a high level of disease development (Fig. 11a).

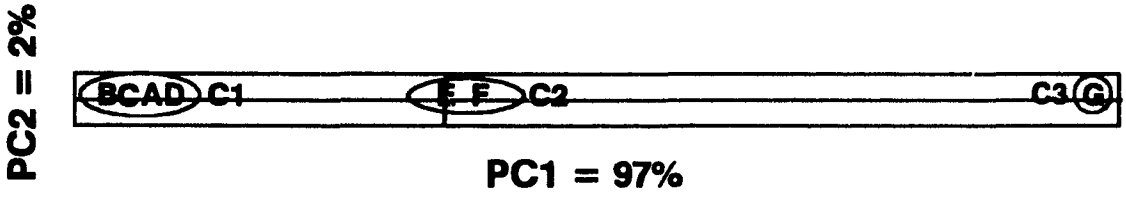
In the field study using mean CPLAD values for each genotype individually at each sampling date as the disease variables, the PC_1 and PC_2 explained 97% and 2% of the variability in the original disease parameters. The component loading of PC_1 was very uniform over all the sampling dates (SD) and values for each were 0.44, 0.45, 0.45, 0.45 and 0.45 for sampling dates SD-1 to SD-5. The plot of PC_1 vs. PC_2 shows very large influence of PC_1 in separating the genotypes and in the separation of the superimposed clusters as well (Fig. 11b). The CA of the 1990 field study gave 3 clusters in which the first cluster grouping contained the oat genotypes OA 712-17, Glen, QO 220.13, and Woodstock, which all denote a high level of partial resistance to *P. coronata* race 264. The second cluster grouping contained 2 genotypes, with QO 574.21 with a high partial resistance and Yamaska with mediocre partial resistance. The last cluster grouping

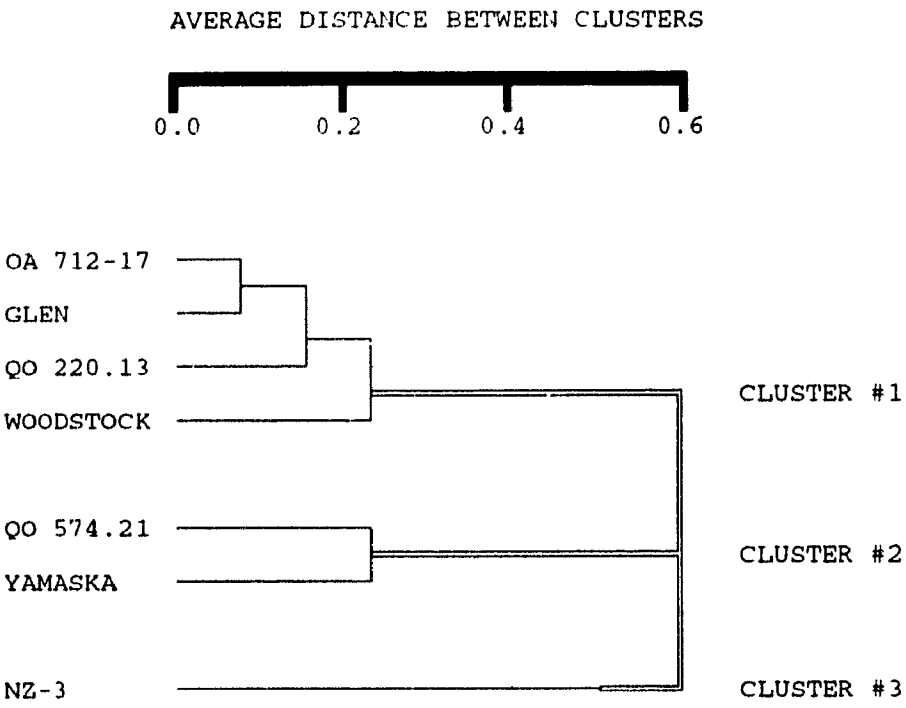
Fig. 11: A Disease progress of *P. coronata* race 264, expressed as the cumulative proportion of leaf area diseased (CPLAD) for oat genotypes within each of 3 clusters during the 1990 field trial. B. The plot of PC_1 vs. PC_2 showing the location of each oat genotype in respect to each other and the results of the cluster analysis superimposed onto the plot equally showing the distances between the cluster groupings relative to each other. C. The dendrogram of the CPLAD values over all five sampling dates for each oat genotype (R^2=0.88).

11A


CLUSTERS
C1
C2
C3
0.5
0.3
0.2
0
CPLAD
SD-1
SD-2
SD-3
SD-4
SD-5
SAMPLING DATES

11 B


PC2 = 2%
BCAD
C1
E F
C2
C3
G
PC1 = 97%


AVERAGE DISTANCE BETWEEN CLUSTERS
0.0
0.2
0.4
0.6
OA 712-17
GLEN
QO 220.13
WOODSTOCK
QO 574.21
YAMASKA
NZ-3
CLUSTER #1
CLUSTER #2
CLUSTER #3

contained only the oat line NZ-3 which is known to have a low partial resistance to *P coronata* race 264 (Fig. 11c).

DISCUSSION

The breeding line OA 712-17 had the smallest TOTSPO and SPO/LES for both trials in the growth bench study. In general QO 220.13 and Glen performed better in the growth bench experiments than Woodstock and QO 574.21 but were very similar under field conditions. The MLP did not vary substantially among the oat genotypes in both trials of the growth bench experiment.

The disease parameters in the growth bench study were very uniform in loading the first principal component which explained most of the variability in both trials. The TOTSPO, MAXLES and SPO/LES had good component loading but the MLP had only a small influence on PC_1. The principal component plots as well as the CA dendrograms gave 3 clusters for both trials of the growth bench experiment. In both instances, the first and second clusters contained only oat genotypes with high levels of partial resistance. The third cluster contained a mixture of oat genotypes with different levels of partial resistance but these were the same in both instances containing the oat genotypes Woodstock (first trial only), QO 574.21, Yamaska and NZ-3. The use of these disease parameters under growth bench conditions tended to underestimate the resistance of the

oat genotypes with high partial resistance but this is probably due to the small number of oat genotypes used in the experiments.

The disease parameters in the field trial (CPLAD values for different sampling dates) were practically identical on their loading of the first principal component which explained almost all the variation among the disease parameters. This was probably due to the fact that the CPLAD parameter on different sampling dates is actually a repeated measure of the same parameter over time. The principal component plot and cluster analysis gave 3 clusters of which the first cluster had four oat genotypes (OA 712-17, Glen, QO 220.13 and Woodstock) and the second cluster had QO 574.21 grouped with Yamaska, due to the better performance of Yamaska in the field on the last two sampling dates.

When comparing the results from the field and growth bench studies, the growth bench results showed more variation than expected in some of the genotypes but this is probably due to the lack the polycyclic effect of *P. coronata* that occurs in the field, which can amplify disease parameters such as latency period and sporulation. Therefore the sporulation parameters quantified from the oat genotypes were the most important in selecting for partial resistance under controlled environmental conditions even though they were tedious to collect.

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GENERAL DISCUSSION AND CONCLUSION

The primary objective of the work reported here had was to screen oat cultivars and breeding lines for their levels of partial resistance to crown rust (*Puccinia coronata*) race 264 under field and growth bench conditions and to select a group of oat genotypes with high levels of partial resistance to crown rust race 264. These oat genotypes selected will then be used in the Macdonald Campus of McGill University oat breeding program.

The selection of oat cultivars and genotypes under field conditions has been shown to be possible based on the multivariate analyses such as principal component and cluster analyses of the disease parameters. Under field conditions, a simplified sampling method using the flag leaf as the main sampling entity on two sampling occasions, when the oat plant reaches the Zadoks growth stages 53 and 85. This method gave very similar results in selecting highly partially resistant oat genotypes when compared to sampling based on 5 leaves after 5 to 6 samplings. When using this simplified method it is helpful to use known races of crown rust so that the effective and ineffective host genes can be used for later comparative studies. It is also important to use some check cultivars with known levels of partial resistance so that these could be used as reference points when conducting the multivariate analyses.

In previous work on other cereal rusts, it was seen that in some cases such as in barley and wheat that interplot interference occurred when small plots were used.

Parlevliet (1988) suggested that in barley underestimation of partial resistance could be as high as 14 to 30 times may occur when using 1.5m plots. In the present investigations where two field studies were conducted, one using 3.8 x 1.0m plot and the second using 5.0 x 5.0m plot, interplot interference did not seem to be an important factor in the selection of partial resistance to crown rust.

The selection of partial resistance under growth bench conditions has also been possible in this work by using two combinations of disease parameters (mean latency period, total lesions/leaf, lesion area; total sporulation, sporulation/lesion, mean latency period, total lesions/leaf). Both the combinations of disease parameters used gave similar results but seemed to underestimate the partial resistance characteristics of some oat genotypes when comparing the results to those obtained from the field screenings. This is likely due to the fact that partial resistance encompasses many factors including genotypic and phenotypic elements (sparse growth habit in the cultivar Glen) as well as excluding the polycyclic nature of *Puccinia coronata* that occurs only under natural field conditions.

Reference

Parlevliet, J.E. 1988. **Strategies for the utilization of partial resistance for control of cereal rusts**. Breeding strategies for resistance to the rusts of wheat. CIMMYT. Mexico.pp 48-62.

CLAIM OF ORIGINALITY

The following point describes the original contribution to knowledge:

The partial resistance in oat cultivars and breeding lines here were not quantified in detail before. The general approach employing multivariate analysis to group oat genotypes based on there resistance to crown rust has never been reported so far. A simplified field screening method for the selection *Puccinia coronata* by using the flag leaf only as the main sampling unit on two sampling occasions which are when the oat plant reaches the Zadoks growth stages 53 and 85.

SUGGESTIONS FOR FUTURE RESEARCH

Using the selection methods developed in this work, breeding lines with high levels of partial resistance from different parental origins could be mixed together to produce multiline cultivars. In this way both the advantages of partial resistance and the physical barrier effect that occurs with multilines can be exploited.