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Iowa State University, Ph.D., 1972
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Factors influencing survival among four competing races of *Puccinia coronata avenae*

by

Elkin Bustamante-R

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Botany and Plant Pathology
Major: Plant Pathology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1972

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INTRODUCTION

Food demanded by human beings increases as population and income per capita increase. Demand for food by an exploding world population has pressured man to grow better crops and use available land in more intensive crop production. Expanding and intensifying production have not only destroyed or endangered the ecosystems that existed prior to the cultivation of homogeneous crops, but also have established new and "foreign" host-parasite systems.

Technology in agriculture also has had the tendency to simplify crop populations to get better quality and yield in a given environment with pure-line cultivars and single-cross hybrids. This, of course, has greatly reduced host diversity and the small number of really different cultivars have, in turn, homogenized the pathogen population that, in turn, has caused destructive epiphytotics. Several workers, concerned with this vicious circle, have started to move in other directions trying to reinstate some intraregional diversity through multiline cultivars, interregional diversity through gene deployment, or to coexist with the pathogen without losing much in yield by using cultivars with horizontal resistance or tolerance (Browning and Frey, 1969; Browning, 1971; Hooker, 1967; Simons, 1969; Watson, 1970).

To place these approaches on a sound scientific basis, new information is required about fungus behavior and evolutionary patterns that will facilitate understanding of ways pathogens evolve and keep ahead in their struggle with the host. Studies on the nature and inheritance of virulence and aggressiveness have been the basis for elucidating fungus evolution.

Two hypotheses have dominated the understanding of the interacting suscept-pathogen system; the first, Flor's (1942), gene-for-gene hypothesis, states that "during parallel evolution host and parasite developed complementary genic systems." Genetic studies that led to the hypothesis also showed that virulence usually is inherited as a recessive trait. The second, Van der Plank's (1968) hypothesis of stabilizing selection, states that a simple race (a race with no or few unnecessary genes for virulence) is the fittest to survive. Flor's work has been confirmed by studies with several host-pathogen systems. The Van der Plank hypothesis, however, although it has been supported in some cases, has been inconsistent with other results. Currently, it probably is the most controversial hypothesis in plant pathology.

My thesis, in part, is an attempt to test this hypothesis. It is a study of the behavior of four crown rust (*Puccinia coronata* Cda. var. *avenae* Fraser & Ledingham) isolates competing for four generations on a common oat (*Avena byzantina* L. 'Bond') suscept, at different inoculum concentrations and temperature regimes under plant growth chamber conditions. My goal is to contribute to the understanding of the possible factors that govern competition and aggressiveness in the crown rust-oat system and, hopefully, to disclose some of the implications of these factors in the fitness of the fungus to survive.

REVIEW OF LITERATURE

Competition and Aggressiveness--General Concepts

Competition, as a process of struggle among different fungus races to occupy host tissue, is important under field conditions only when the amount of disease is higher than about 20%. However, differences in fitness among races are controlled more by differences in infection rates during initiation of the disease than by direct struggle among strains of the same pathogen (Van der Plank, 1968).

Virulence, as measured by differential interactions of different cultivars, gives some races relative competitive advantages on the cultivar attacked. However, if different races can be ranked by their different interactions on the same suscept, the difference is believed due to differences in aggressiveness (Van der Plank, 1968). Aggressiveness is a term that describes the pathogenic characteristics that allow one race to predominate over others on susceptible cultivars (Browder, 1965). "Competitive ability" and "survival ability" are considered synonymous with aggressiveness; however, they should have different meanings in some circumstances (Katsuya and Green, 1967).

Pathogenicity is considered to include both aggressiveness and virulence (Van der Plank, 1968; Watson, 1970). Virulence is considered by Van der Plank (1968) to be inherited oligogenically and aggressiveness polygenically, and that differences in aggressiveness are quantitative more than qualitative. One of the basic assumptions in competition is that the simple race of the pathogen is the fittest to survive on simple cultivars (Van der Plank, 1963, 1968). A simple race is one with no or few unnecessary genes for virulence, and a simple host is one with no or few genes for vertical resistance. Hence, Van der Plank considers it axiomatic that pathogens that have more genes for virulence have less fitness than those with fewer genes for virulence. Considerable field and greenhouse data support this assumption (Aslam and Browder, 1971; Browning and Frey, 1969; Leonard, 1969; Van der Plank, 1963, 1968; Watson, 1958). Van der Plank (1968) assumed that there is no evidence for a positive correlation between aggressiveness and virulence, which means that an increase in virulence will not necessarily bring an increase in aggressiveness.

Race Surveys and Fitness of Crown Rust Races

Browning and Frey (1969) described shifts in the populations of crown rust and other oat pathogens in response to shifts in oat cultivars grown in the USA. They discussed the apparent failure of vertical resistance since its use caused a corresponding shift of virulence in the pathogen, feeding in that way a "vicious circle." A similar opinion was expressed by Watson (1970). Crown rust data that tend to support Van der Plank's theory show that the most virulent race of crown rust, race 264A, is not the most prevalent race in the rust population, even though it has been present since 1955 (Michel and Simons, 1966; Simons, 1970). Michel and Simons (1971) showed that race group 290, with fewer genes for virulence than race group 264A, accounted for a higher percentage of the crown rust population in North America. However, their more recent data show that race group 264B, intermediate in virulence between race groups 326 and 264A, is coming to be the most prevalent race group in the crown rust population. This result and data from studies with other fungi (Brown and Sharp, 1970; Katsuya and

Green, 1967; Loegering, 1951; Martens, Mackenzie and Green, 1970; Nelson, Mackenzie, and Scheifele, 1970; Thurston, 1961; Watson, 1970), complicate the validity or generality of Van der Plank's theory of stabilizing selection. Watson (1970), after analyzing several works, concluded "that if a gene for virulence has no deleterious effect and is associated with genes for aggressiveness and survival ability in a well-adapted strain, it may remain in the population regardless of whether it is necessary or not."

Ways of Measuring Competition and Aggressiveness

Broyles (1955) considered that the survival potential of different biotypes of *Puccinia graminis* should be measured using factors like percentage germination and rate of spore increase. Four factors were described by Van der Plank (1968) to determine fitness of a pathogen: 1) the number of spores that will germinate and infect the plant, 2) the period between inoculation and the eruption of uredia, 3) spore yield, and 4) duration of spore production. Simons (1970) added to these factors the rapidity of telial formation. The viability and longevity of spores also are important in measuring the fitness of a pathogen (Brown and Sharp, 1970).

Torres (1966) found an important factor for determining fitness in *P. coronata* to be the evenness of spore production during the spore production period. He observed that some races had a more regular distribution of spore production than others, and that different races had their highest spore yield at different days after inoculation. He concluded that the race with the most uniform distribution will have advantages in a fluctuating environment. This factor should be considered in experiments where the weight of uredospores is related to the size of uredia (Katsuya and

Green, 1967). It also could explain the result of Ogle and Brown (1970) that the length of time between two successive inoculations could influence which *P. graminis tritici* strain predominated in the mixture.

Effect of Inoculum Concentration on Mixtures of Fungus Isolates

The concentration effect on mixtures was discussed by Katsuya and Green (1967) in relation to a study with *P. graminis tritici* races 56 and 15B-1 (Can). Race 56 predominated when infection was light but when Little Club was heavily infected, race 15B-1 (Can) was more prevalent.

Effect of Temperature on Mixtures of Fungus Isolates

Peturson (1930) considered that temperature can affect pustule formation, accelerating this process at high (25C) compared to low (14C) temperatures. He found a temperature of 21C optimum for crown rust development and emphasized the importance of using that temperature in race identification. Simons (1954) observed that the effect of temperature on the reaction to crown rust varied with different maturity stages of the cultivar. Zimmer and Schafer (1961) concluded that the interaction between the oat cultivar Glabrota and crown rust was temperature labile giving a resistant reaction at 14C and a susceptible one at 25C.

Katsuya and Green (1967) considered that temperature could be important in the outcome of the associated growth of *P. graminis tritici* races 15B-1 (Can) and 56. A low temperature favored race 15B-1 (Can) and a higher temperature race 56. Working with *P. striiformis*, Brown and Sharp (1970) found temperature effects on the proportion of Bonner's Ferri isolate (BFI) and the albino Bozeman isolate (BIa) in mixtures. Isolate (BFI) increased after each generation in 15/24C and 2/18C temperature regimes when the original mixture had 23% of isolate (BFI); however, isolate (BIa) was reduced to less than 3% at the low temperature regime after the first generation. At the high temperature regime, isolate (BIa) decreased slowly from the original 27%. Aslam and Browder (1971), working with _P. recondita_, observed that mixtures of three cultures were unaffected by either temperature or photo-period.

Leaf-Wetness Duration, Infection, and the Spore Production Process

Leaf-wetness duration and the resultant infection Bromfield (1970) indicated that attempts to correlate germination with infection potential were disappointing because the total infection process is much more complex than germination, the first step. Germination is usually higher than 90% with fresh inoculum (Bromfield, 1967; Loegering, 1951). Old spores often have sufficient vigor to produce germ tubes but lack sufficient energy to develop the subsequent infection structures (Bromfield, 1967).

Loegering (1951) did not find any difference in germination among the competing _P. graminis_ races he studied. Broyles (1955) observed some differences among results of different germination tests but these were at random and should not be considered significant over a long period.

Ogle and Brown (1971) pointed out that differences in the rate of development during pre-penetration and penetration stages should give _P. graminis tritici_ race 21-2,7 a competitive advantage over race 21-2,3,7. This assumption was made thinking that the chances of survival would be higher in the strain that germinates, grows, and penetrates faster since that strain would be more likely to avoid desiccation before penetration. Marland (1938) observed infection with *P. coronata* after a 5-hr period at the optimum temperature of 17-27C. The infection process took longer at 30C or at temperatures lower than 17C.

The period between inoculation and the eruption of uredia is considered important in survival ability. Watson (1970) observed that in race 34 this period was shorter than that of other *P. graminis tritici* races. In the same way, the period between inoculation and the eruption of uredia was shorter for race 56 than for race 15B (Browder, 1965) or for race 15B-1 (Can) (Katsuya and Green, 1967). Loegering (1951) and Ogle and Brown (1971) did not find any differences in this period in the *P. graminis tritici* races they investigated.

Size of uredia and duration of spore production Torres (1966) found that *P. coronata* race 290 formed larger uredia on Markton oats than on Clinton or Cherokee; however, race 290 yielded more uredospores on Clinton than on the other cultivars. He also observed that race 216 formed bigger uredia on Cherokee and Markton than on Clinton; however, spore yield was greater on Clinton than on Cherokee or Markton. Singh (1971) concluded that the uredia of *P. coronata* race 326 were longer than those of race 290 ten days after inoculation.

P. graminis tritici race 15B-1 (Can) formed uredia larger than those of race 56; however, race 56 grew more rapidly and produced twice as many spores (Ogle and Brown, 1971). They also observed a good correlation between uredial size and the number of spores produced.

Torres (1966) indicated that uredospores of *P. coronata* race 216 were not distributed regularly during the spore production period on Cherokee but that it peaked significantly 23 days after inoculation. So, the tolerant Cherokee placed race 216 at a competitive disadvantage in a fluctuating environment. Browder (1965) reported that the peak for sporulation of *P. graminis tritici* races 15B and 56 was found between 16 and 24 days after inoculation. Similar results were noted with races 6F and 7A of *P. graminis avenae* (Leonard, 1969). Broyles (1955) considered that, under field conditions, the effective life of *P. graminis* uredia was only 17 days.

Telial formation Watson (1958) expressed that the characteristic of early teliospore formation should be tied to a lower chance of survival. Simons (1970) considered the rapidity of telial formation to be a measure of aggressiveness.

Oil and Moisture Effects on Germination

Sharp and Smith (1952) reported that the germinability of vacuum-dried uredospores was increased by exposing them to an atmosphere saturated with water vapor. The hydration was equally effective in light and darkness (Sharp, 1965). These results have been corroborated by other workers (Bromfield, 1967; Wise and Daly, 1967). Wise and Daly (1967), however, considered that hydration under dark conditions reduced germination in spores having high levels of germination. Bromfield (1967) suggested that there is more to the hydration process than just the physical sequestering of water molecules. Fischer and Melching (1969) found that *P. graminis tritici* uredospores, exposed over glycerol-water solutions at different relative humidities, had an optimum germination level in the 92-98% relative humidity range, but they observed a marked reduction between 74 and 84%. They considered that some physiological process or processes are affected at that vapor pressure range. Strobel (1965) concluded that hydration of P. striiformis uredospores started a series of dynamic physiological events that are associated with an increase in spore germination. He noted that hydration should serve to increase protein activation or cell fluidity that control mitochondrial migration.

Rowell (1956) indicated that spores in an oil carrier remained viable longer than dry spores and that they did not germinate until oil was in contact with free water. Thus, spores were unaffected by suspension in oil for the several hours required to inoculate all plants in a field. The use of oil prior to hydration restored slowly the delicate water balance of the spores avoiding the possible lethal injuries that may occur when spores are transferred directly to water.

Staples et al. (1971), working with spores hydrated for 16 hr by floating them on a water surface at 4C, observed that uredospores germinated on moist collodion membranes containing paraffin oil were short, thick, and differentiated. The same result was obtained by Maheshwari, Hildebrandt, and Allen (1967). Simons (1970) reported that in some experiments petroleum jelly and paraffin oil in contact with water stimulated germination.

MATERIALS AND METHODS

General Procedure

Inoculum One monouredial culture of each of four races (216, 264A, 264B, and 326) of *Puccinia coronata* Cda. var. *avenae* Fraser and Ledingham was used. These races have been classified by Michel and Simons (1966, 1971) into four groups:

1. Race group 216 includes races 213A and 216 and is virulent on differential cultivars Bond and Victoria. Race 216 was the predominant race in 1955 and typifies the so-called "old group" of non-Landhafer races. It is of negligible importance at the present time.

2. Race group 290 includes races 290, 295, 321, and 326 which attack Bond and Landhafer but not Trispernia. This group is close to race group 264B in some respects, but the inability of race group 290 to attack Trispernia and Bondvic distinguishes both groups. Race group 290 was predominant in the 1960's but it is now giving way to race group 264B.

3. Race group 264B includes only race 264B. It is similar to race group 264A on the standard set of differentials, but cultivars like Ascencao and X-421 condition resistance to race 264B. Race 264B, first identified in 1963, has become the predominant race in recent years.

4. Race group 264A includes only race 264A. It attacks all differentials except Saia. It differs from race group 264B in its ability to attack some genotypes like cultivar X-421 not included in the standard set of differentials. Race 264A has the widest range of virulence and at one time was rated virulent on seedlings of all known hexaploid oat cultivars.

My rust cultures were established by removing spores from individual uredia with a sterile needle and transferring them to seedling leaves of the oat cultivar Bond planted in 3-inch pots. Plants in each pot were covered with a lamp chimney isolation chamber to avoid contamination. The increase of inoculum took three consecutive uredial generations and the spores produced in each generation were collected by carefully shaking the seedling leaves on aluminum foil in still air. This method of collection was standard during the different experiments.

My culture of each race represents only the increase of one monouredial isolate. Its behavior in my experiments will not necessarily reflect the way the many other possible biotypes of that race would behave. For convenience, however, isolates hereafter will simply be called "races" to facilitate the description of results and discussion.

Cultivars Table 1 shows the cultivars used in this study, the crown rust resistance genes they carry, and the reactions of each cultivar to the four races I used. Inoculum was increased initially on *Avena byzantina* L. 'Bond' that served also as "universal" suscept for the races and race mixtures each subsequent generation. Note that, although Bond has crown rust resistance genes *Pc*-3 and *Pc*-4, it seems equally susceptible to all isolates in this study. Thus, the studies of inoculum concentration and temperature effect were made on Bond, while isolines C-649 and X-421 (both *A. sativa* L.) served as index cultivars to determine the ratio of the different races in the different mixtures after each generation on Bond. Bond, C-649, and X-421 were chosen because of their relationship and because, respectively, they carry progressively more resistance genes. There is a question whether C-649 and X-421 carry genes *Pc*-3 and *Pc*-4 (Table 1). However, due to the breeding system used to develop these cultivars, the

Table 1. Data on cultivars of oats and races of *Puccinia coronata avenae* used in this study

Cultivar or Isoline	C.I. No.	Resistance genes carried[a]	Reaction[b] to races 216	264A	264B	326
Cultivars used in all experiments						
Bond	7004	*Pc*-3,*Pc*-4	S	S	S	S
C-649	7555	*Pc*-3?,*Pc*-4?,*Pc*-5	R	S	S	S
X-421	- [c]	*Pc*-3?,*Pc*-4?,*Pc*-5,*Pc*-52	R	S	R	R
Cultivars used to identify races						
Anthony	7001	not determined	S	S	S	S
Victoria	7002	*Pc*-2,*Pc*-11,*Pc*-12	S	S	S	S
Appler	7003	*Pc*-1	S	S	S	S
Bond	7004	*Pc*-3,Pc-4	S	S	S	S
Landhafer	7005	*Pc*-5	R	S	S	S
Santa Fe	7006	*Pc*-6,*Pc*-7,*Pc*-8,*Pc*-21	R	S	S	S
Ukraine	7007	*Pc*-6,*Pc*-9	S	S	S	S
Trispernia	7008	*Pc*-6d	R	S	S	R
Bondvic	7009	not determined	R	S	S	R
Saia	7010	*Pc*-15,*Pc*-16,*Pc*-17	R	R	R	R

[a] Simons *et al.*, 1966 and M. D. Simons, Department of Botany and Plant Pathology, Iowa State University, Ames, Iowa 50010, private communication, 1972.

[b] R = Resistant, S = Susceptible.

[c] Pedigree: C.I. 7555^6 x Avena sterilis L. 'Wahl No. 2.'

probability is high that they do. The standard set of differential cultivars (Simons, 1970) was used each inoculation to double check each of the isolates.

Bond was grown 10-12 plants/3-inch pot in greenhouse soil (1 sand: 1 muck: 2 field soil). Cultivars C-649 and X-421 were planted on opposite sides of the same 3-inch pot, using 10-12 plants/cultivar. The 10 standard differential cultivars were grown 8-10 plants/cultivar in 11 x 15-inch plastic flats. Approximately 10 days after planting, primary leaves were inoculated.

Inoculation A spore settling-turntable tower (manufactured by the Iowa State University Instrument Shop from plans supplied by Dr. J. S. Melching, Ft. Detrick, Maryland) was used for the inoculation. Some modifications (designed by Dr. J. A. Browning) include a central station for oil inoculation, an air-flow meter, a device for continuous agitation of uredospores in oil, and an atomizer, timer, and solenoid valve to allow a known quantity of spores to be delivered in a known volume of air in a programmed amount of time. The turntable speed was 20 rpm, the solenoid timer was adjusted to 9 sec, and air flow on the manometer was kept at 11.5. The air agitator was open until spores remained suspended.

The plants were placed on the center turntable and the primary leaves sprayed with uredospores suspended in Mobilsol 100[1/] (Rowell, 1957). The plants were allowed to dry for at least 10 min, then they were atomized with distilled water and kept over night in a moist chamber in a 21C air

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[1/] An iso-paraffinic non-phytotoxic oil available as product code No. 754-259 from the Mobil Oil Co., 7280 Caldwell Avenue, Niles, Ill.

conditioned lab. Next morning the moist chamber was opened and the plants allowed to dry gradually in diffuse light for four hr. Then the plants were transferred to the place provided for a given experiment. X-421 and C-649 were kept in a Plant Growth Lab[1] at 24 ± 1C to avoid differences due to temperature (Simons, 1954). The light intensity of 2,200 ft-c was supplied by Sylvania VHO cool-white fluorescent bulbs for a 14-hr photoperiod.

Experimental design I used a randomized block design and measured two characters on each plant: the number of uredia/plant of the different races and the ratio of resistant to susceptible infection types in the different mixtures. An analysis of variance was run of each character and also for comparing the performance of cultivars X-421 and C-649 in each of the four experiments (Table 2). The number of replications per experiment was determined by the number of lamp chimneys available.

A third character, spore germination, was measured after each inoculation for all treatments. The germination percentage was determined 24 hr after a spore-oil suspension was deposited on 0.1% water agar in petri dishes. A drop of cotton blue was placed in the center of the suspension to stop germination after 4 hr in the germination tests for the four generations of experiments 3 and 4. The data recorded in experiments 3 and 4 were percentage of germination and length of germ tubes developed after 4 and 24 hr. All spore germination tests were run in darkness in a 21C incubator.

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[1] Model No. PGW-132 manufactured by the Percival Manufacturing Co., Boone, Iowa.

Table 2. Sources of variation and degrees of freedom in the analyses of variance for the four experiments

Source	Experiment 1	 2	 3	 4
Replication	2	2	1	1
Treatment	(23)	(35)	(143)	(215)
Race	3		3	
Mixture		5		5
Generation	1	1	3	3
Temperature			8	8
Concentration	2	2		
C x R or M	6	10		
G x R or M	3	5		
G x C	2	2		
G x R or M x C	6	10		
G x R or M			9	15
T x R or M			24	40
G x T x R or M			72	120
G x T			24	24
Error	66	70	143	215
Total	71	107	287	423

Other data recorded were the period between inoculation and the eruption of uredia, size of sporulating area, duration of spore production, and telial formation. Half-lives of my race isolates, in the concept of Van der Plank (1968), were calculated for the different race mixtures in experiment 4. All experiments are outlined in Table 3.

Effect of Inoculum Concentration on Mixtures of Fungus Isolates

Three inoculum concentrations, low, medium, and high, were used to inoculate Bond with the four races (experiment 1) and six mixtures (experiment 2). After inoculation the seedlings were kept in a Plant Growth Lab at a 26/17 ± 1C day/night temperature regime and 2,200 ft-c. Experiments were run on Bond for two generations. Inoculum concentrations, use made of cultivars X-421 and C-649, and temperatures inside the lamp chimneys are discussed with the results.

Effect of Temperature on Mixtures of Fungus Isolates

Three different day/night temperature regimes (21/12, 26/17, and 32/19 ± 1C) were applied to Bond seedlings inoculated with the four races (experiment 3) and six mixtures (experiment 4) used in the inoculum concentration experiments. Three Plant Growth Labs were calibrated for the temperature regimes selected and a 2,200 ft-c light intensity was maintained during a 14-hr photoperiod. The inoculum concentration was that of the medium treatment in experiment 2. Experiments 3 and 4 were run for four generations. Details of temperature selection are included with the results.

Table 3. Experiment number and study, number of generations[a] on the common suscept Bond, index cultivars, and traits measured in the nine experiments run with four races or six mixtures of *P. coronata avenae*

Exp. No.	Study	Generations on Bond	Index Cultivar	Inoculum		Traits measured
				Races	Mixtures	
1	Inoculum concentration	two	X-421 C-649	four	-	Number of uredia/leaf on Bond and index cultivars, and spore germination
2	Inoculum concentration	two	X-421 C-649	-	six	Number of uredia/leaf on Bond, R/S ratios on index cultivars, and spore germination
3	Temperature	four	X-421 C-649	four	-	Number of uredia/leaf on Bond and index cultivars, and spore germination
4	Temperature	four	X-421 C-649	-	six	Number of uredia/leaf on Bond, R/S ratios on index cultivars, and spore germination
5	Leaf wetness duration	one	-	four	-	Number of uredia
6	Time until eruption of uredia	four	-	four	-	Period between inoculation and eruption of uredia and duration of spore production
7	Uredial size	one	-	four	-	Uredial size
8	Telial formation	four[b]	-	four	-	Telial formation
9	Oil and moisture	-	-	four	-	Spore germination

[a] Inoculum collected in each generation of Bond was used as the inoculum for producing the next generation on Bond and as the inoculum for the index cultivars.

[b] Telial formation was checked on all oat cultivars described in Material and Methods.

Leaf-Wetness Duration, Infection, and the Spore-Production Process

Bond was inoculated with the four races and placed in a moist chamber for 4.5 or 5.5 hr. One experiment was kept in the greenhouse and the other in a Plant Growth Lab.

A set of differential cultivars was inoculated with the four races and kept under the same temperature regimes used in temperature experiments 3 and 4. Observations about the period between inoculation and the eruption of uredia, size of uredia, duration of spore production, and extent of telial formation were taken.

Oil and Moisture Effects on Germination

Spores of the four races were collected from the Plant Growth Lab where the temperature was controlled at a 26/17 ± 1C day/night regime. The spores were stored in a desiccator for 24 hr. Later spores were placed in thin layers in a moist chamber for rehydration. A drop of Mobilsol 100 was added to the samples at different times after the spores began rehydration, and germination tests were run to determine the effect on germination of oil, rehydration, and the interaction of the two.

RESULTS

Preliminary Investigations

I needed to examine several problems initially. The first was to learn the effect of lamp chimney isolation chambers on oat seedlings and to measure any differences they caused in temperature. Another problem was to select isogenic lines to use in identification of the different races in each mixture. Also, I examined data relevant to changes in the host population.

Lamp chimney effects   The average temperature ratios outside/inside the lamp chimneys were measured with laboratory thermometers and were as follows: 29/33, 27/31, 24/27, 18/20, 15/17C. Thus, temperatures increase inside lamp chimneys 3 to 4C at high temperatures (24-29C) but only 2C with lower temperatures (15-18C).

Humidity inside the lamp chimneys was high especially during the dark period when free moisture condensed. This allowed the rust fungus to cycle within the moist isolation chamber and enabled me to learn that the period between inoculation and the eruption of uredia of the four races was two days shorter on material held under lamp chimneys. Since this period differed among races I collected spores 14 days after inoculation to avoid collecting spores from secondary uredia.

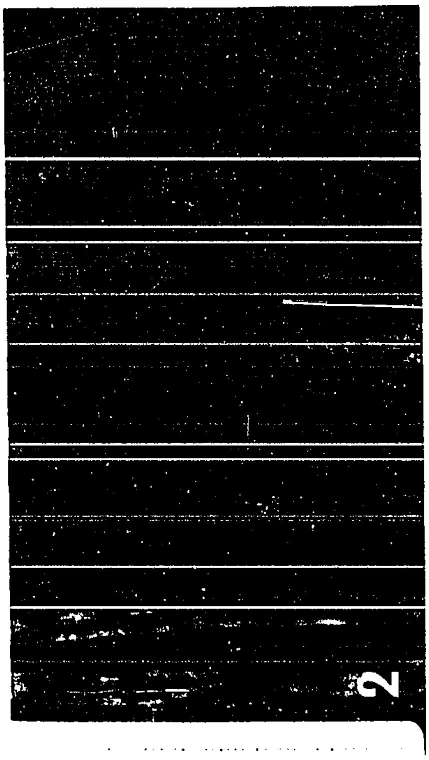
Cultivar selection   Four isogenic cultivars (X-421, X-465, X-765, and C-649) were tested to check their reactions to the four races selected for my experiments. X-421 was selected to differentiate race 264A from the other races in mixtures. C-649 distinguished clearly between race 216 and the others. A cultivar to distinguish between races 264B and 326 was not available so mixtures of these races were dropped. I observed that my isolate of race 326 induced development of a pink halo surrounding the type-4 uredia on C-649; however, this was inconsistent and on the basal third of the leaf the lesions were indistinguishable from those produced by my isolate of race 264B.

The infection types produced by the different races on X-421 and C-649 are shown in Fig. 1-4. Note in Fig. 1 that race 326 has difficulty infecting X-421 compared with races 216 or 264B. Race 216 attacks C-649 mildly and the infection type produced is $2^{\pm}$, while races 264A, 264B, and 326 produced infection types 4^{-}, 4^{+}, and 4^{+}, respectively.

Oat population changes The oat population changes when new cultivars with improved agronomic type and yield are released and grown commercially. This is linked very commonly to the evolution of the most serious pathogen of the crop. In studying these changes, I could not obtain data for the total acreage of each cultivar planted annually in Iowa, so I used the data for acreage planted for certified seed production in Iowa from 1966-1970 (Iowa Crop Improvement Association, 1966, 1967, 1968, 1969, 1970), which should be a fair estimate of the percentage of each cultivar in the commercial oat population. This information was supplemented with data on the reactions to different groups of crown rust races of the cultivars planted during that period (Browning *et al.*, 1970; Frey *et al.*, 1966, 1968, 1969; Grindeland *et al.*, 1967). These data, presented in Table 4 and Fig. 5, show that cultivars susceptible to races 216, 264A, 264B, and 326, or resistant only to race 216, have almost dropped from the population. Cultivars resistant to races 216 and 326 also are decreasing and only Garland, which is attacked by race 264B, remains with more than 20% of the planted acreage. Multilines represent the group of cultivars that is replacing

Figs. 1-2. Infection types, from left to right, of isolates of crown rust races 216, 264A, 264B, and 326 inoculated onto oat cultivars X-421 and C-649, respectively

Figs. 3-4. Infection types, from left to right, of crown rust race mixtures 216+264A, 216+264B, 216+326, 264A+264B, 264A+326, and 216+264A+264B+326, inoculated onto oat cultivars X-421 and C-649, respectively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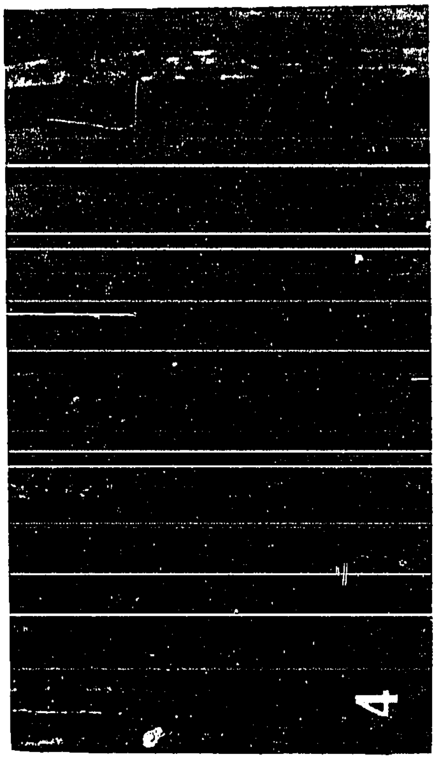
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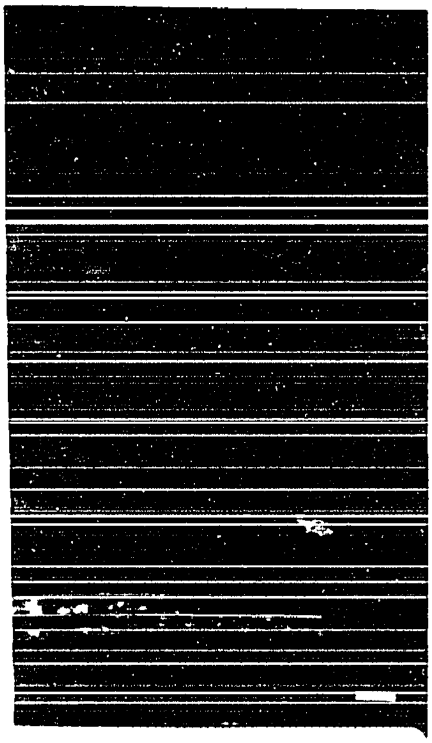

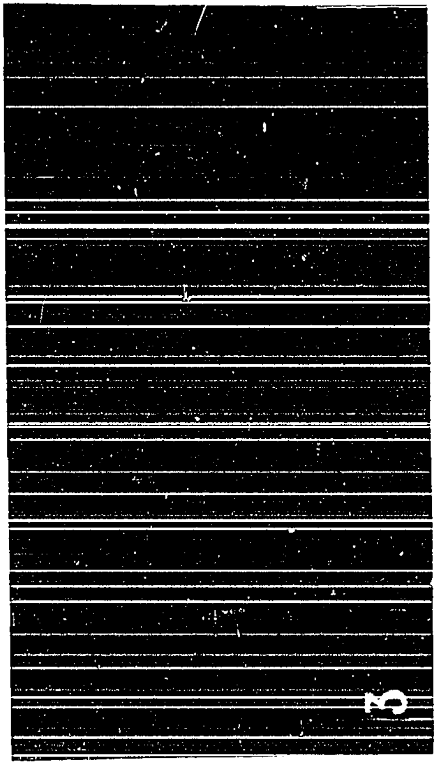
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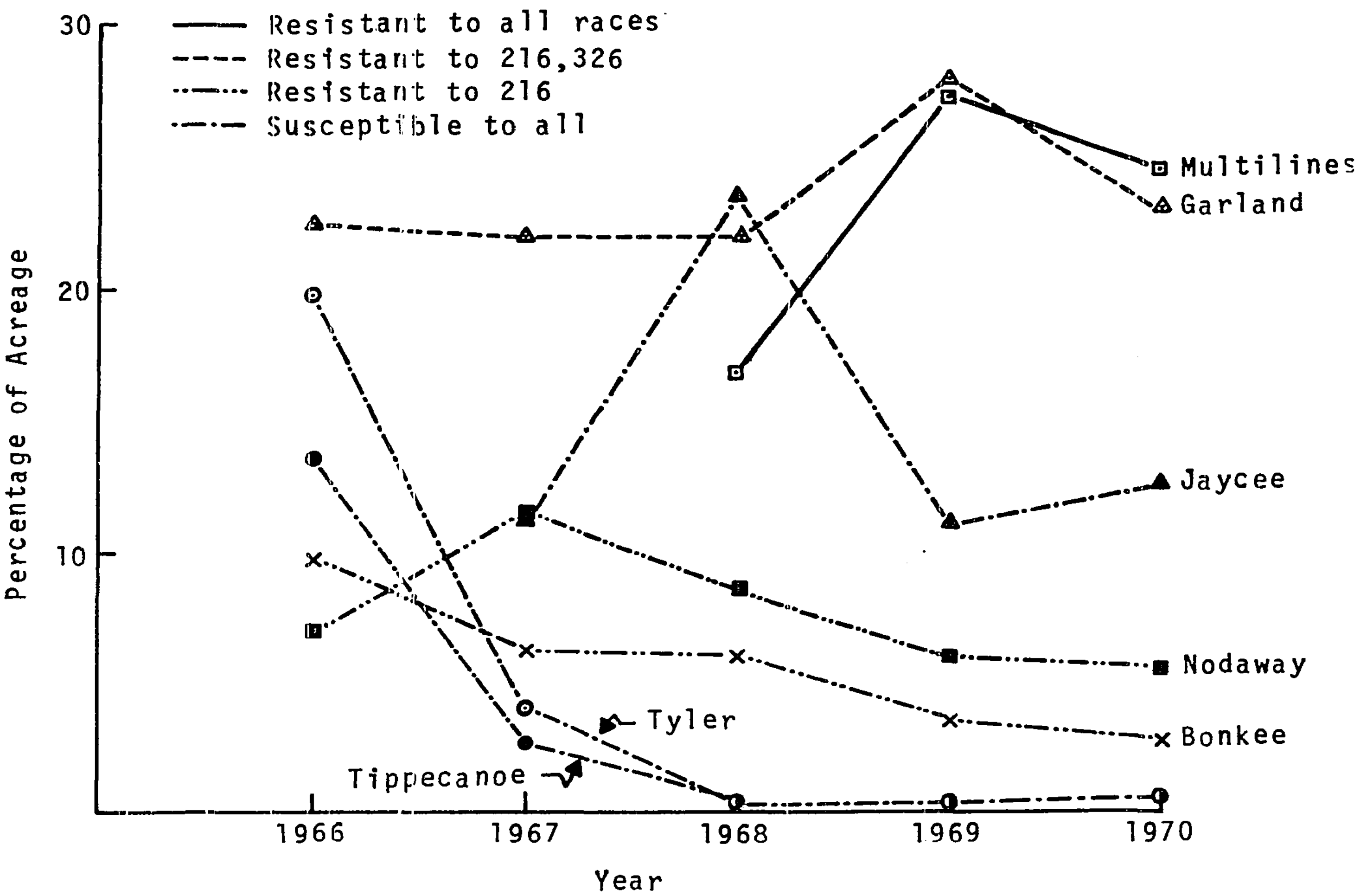
Table 4. Oat cultivars grown for certified seed in Iowa during the 5-year period 1966-1970, their changing reactions to a changing crown rust population, and the percentage of each in the total acreage grown for certified seed

Cultivar	Acres					Reaction[a] to races			
	1966	1967	1968	1969	1970	216	326	264A	264B
Bonkee	9.7	6.2	6.0	3.5	2.9	S	S	S	S
Cherokee	5.4	3.5	0.2	0.2	-	MS	S	S	S
Clintford	4.2	10.0	2.8	3.9	9.7	R	S	S	S
Clintland	1.3	0.7	0.2	0.1	0.1	R	MS	S	S
Garland	22.6	22.1	21.9	28.0	23.0	R	R	MS	S
Goodfield	2.9	1.4	0.2	0.1	-	R	MR	MS	S
Holden	-	0.4	4.0	4.7	9.1	R	R	MS	S
Jaycee	-	11.3	23.5	11.1	12.5	R	S	MS	S
Multilines[b]	-	-	16.7	27.3	24.6	R	R	R	R
Neal	3.0	2.5	1.2	1.1	0.9	R	S	S	S
Nemaha	4.2	4.4	2.6	1.4	1.3	MS	MS	S	S
Nodaway	6.9	11.5	8.6	6.0	5.6	S	MS	S	S
O'Brien	-	7.3	7.8	5.6	6.3	R	MS	MS	S
Portal	-	0.4	1.1	1.1	2.0	R	R	MR	MR
Stormont	5.2	10.4	2.8	4.3	0.7	S	S	S	S
Tippecanoe	13.4	2.7	0.2	0.2	0.4	R	S	S	S
Tyler	19.7	3.9	0.2	0.2	-	R	S	S	S
Total	100	100	100	100	100	-	-	-	-

[a] R = Resistant, S = Susceptible, M = Moderately.

[b] Multiline oat cultivars in two series, early and midseason, were combined for purposes of this analysis.

Fig. 5. Changes in the percentage of the acreage of oat cultivars grown for certified seed in Iowa during the 5-year period, 1966-1970


Resistant to all races
Resistant to 216,326
Resistant to 216
Susceptible to all
30
20
10
Percentage of Acreage
1966
1967
1968
1969
1970
Year
Multilines
Garland
Jaycee
Nodaway
Bonkee
Tyler
Tippecanoe

the material dropped since 1968. These changes are more meaningful when they are related to changes in the major race groups of *P. coronata avenae* collected in the USA from 1966-1970 (Michel and Simons, 1971). According to this information race groups 216 and 290 have decreased in the population and race group 264B has predominated.

Effect of Inoculum Concentration on Mixtures of Fungus Isolates

Experiments 1 and 2 were planned to study the effect of inoculum concentration on the relative survival of races 216, 264B, and 326 (exp. 1) and the following mixtures: 216+264A, 216+264B, 216+326, 264A+264B, 264A+326, and 216+264A+264B+326 (exp. 2). The inoculum concentrations were 10 mg of spores per 7.5 ml of Mobilsol 100 (high concentration), 10 mg of spores per 15.0 ml of Mobilsol 100 (medium concentration), and 10 mg of spores per 30.0 ml of Mobilsol 100 (low concentration). Seedlings of Bond, X-421, and C-649 were inoculated and kept in a Percival growth chamber programmed for a 26/17C day/night temperature regime. Two consecutive uredial generations were increased on Bond. After each, spores were harvested and indexed for the proportion of each race in the population (exp. 2) by the ratio of resistant to susceptible infections on X-421 and C-649, except that the infection type 4^- of race 264A and the 4^+ of race 264B distinguished these races in mixtures on C-649.

Significant differences due to concentration were noted in experiments 1 and 2 for the average number of pustules on Bond for races and mixtures, and for the interaction of concentration x races or mixtures (Tables 5 and 6). The number of pustules on Bond decreased sharply with decrease in spore concentration when Bond was inoculated with races 216, 264A, or

Table 5. Average number of pustules per primary leaf of Bond oats inoculated with four races or six mixtures at three different inoculum concentrations during two generations (exp. 1 and 2)

Race or Mixture	Inoculum concentration		
	High	Medium	Low
216	54	39	26
264A	46	38	13
264B	98	53	20
326	65	50	28
216+264A	94	89	32
216+264B	71	59	32
216+326	64	55	40
264A+264B	103	80	21
264A+326	51	43	30
216+264B+264A+326	99	60	24

Table 6. Mean squares from the analysis of variance of the number of pustules per plant on Bond seedlings (exp. 1 and 2)

Source of variation	Mean square	F
Races and Mixtures	1970.239	60.434 **
Concentration	1257.560	385.642 **
R and M x C	570.787	17.508 **
Error	32.601	

** F value exceeds 1% level of significance.

264B (Fig. 6); however, the decrease was less significant with race 326 or with most of the mixtures in which it was a component. This should account for the interactions indicated and it shows also that different races produced different numbers of pustules. According to results from experiment 1, at low inoculum concentration my isolates of races 216 and 326 should be considered more aggressive than 264A or 264B; however, isolates of races 264B and 326 were the more aggressive isolates at the medium and high inoculum concentrations.

The response of X-421 and C-649 to the four races in experiment 1 were highly significant at all sources of variation except the generation x concentration interaction for X-421 (Tables 7 and 8). The difference among races on X-421 is mainly due to the mild attack by races 216, 264B, and 326 which resulted in a significantly smaller number of pustules in comparison to those of race 264A (Fig. 1). The average pustule number of races 216 and 264B on X-421 showed only minor differences between generations (Table 7). The same races inoculated onto C-649 showed that in the second generation the number of pustules was more than twice that in the first generation. Race 264A, which produced the same pustule type with a similar sporulating area on X-421 and C-649 (Fig. 1 and 2), infected both cultivars with the same intensity in the first generation; however, the number of pustules doubled on X-421 and increased up to five times (high concentration) on C-649 during the second generation (Fig. 7). The difference between generations that accounts for the significance of the race x generation interaction probably was caused by factors that affected the seedlings before inoculation or by the quality of the spores used in the different inoculations. The differences between X-421 and C-649 were highly significant

Fig. 6. Average number of pustules per leaf of Bond oat seedlings inoculated with races 216, 264A, 264B, and 326, and six race mixtures with three spore concentrations (exp. 1 and 2)

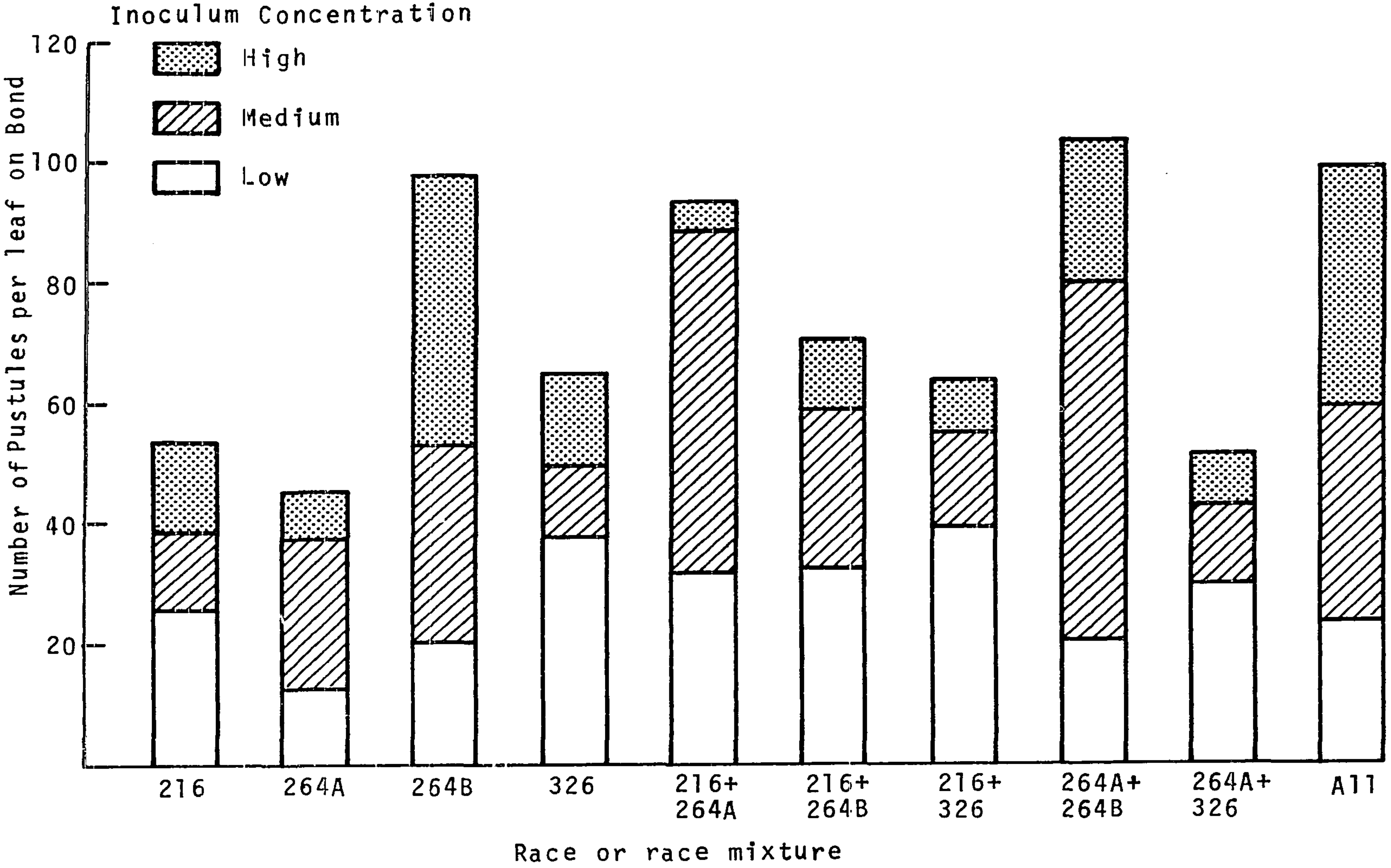

Inoculum Concentration
High
Medium
Low
Number of Pustules per leaf on Bond
120
100
80
60
40
20
216
264A
264B
326
216+ 264A
216+ 264B
216+ 326
264A+ 264B
264A+ 326
All
Race or race mixture

Table 7. Index (average number of uredia) on X-421 and C-649 of two uredial generations of four crown rust races produced on Bond at three inoculum concentrations (exp. 1)

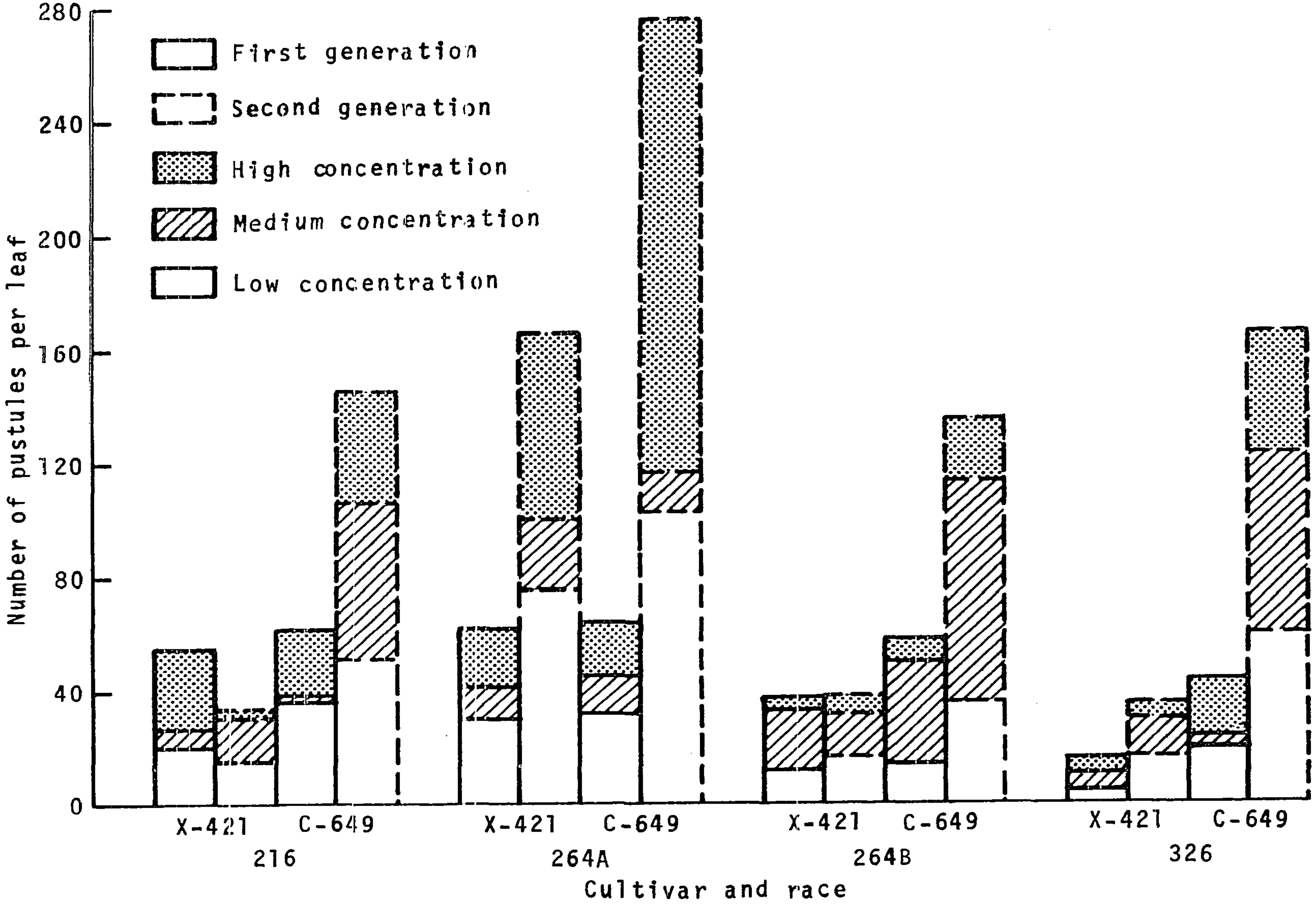
Race	Inoculum concentration	Cultivar and generation X-421 I	X-421 II	C-649 I	C-649 II
216	High	55	30	63	146
	Medium	27	33	38	107
	Low	21	16	37	51
264A	High	52	167	54	276
	Medium	41	99	45	117
	Low	30	76	31	102
264B	High	37	38	58	137
	Medium	33	32	51	113
	Low	13	17	14	37
326	High	17	36	43	166
	Medium	11	30	23	123
	Low	5	16	19	60

Table 8. Mean squares from analyses of the number of pustules of four crown rust races on X-421 and C-649 oats (exp. 1)

Source of variation	Cultivar X-421	Cultivar C-649
Generation	2862.093 **	23297.040 **
Race	2608.300 **	4908.332 **
G x R	7658.371 **	35038.660 **
Concentration	5397.700 **	32825.470 **
G x C	152.680	3913.748 **
R x C	645.121 **	1501.100 **

** F value exceeds 1% level of significance.

Fig. 7. Average number of pustules per plant on index cultivars X-421 and C-649 inoculated with races 216, 264A, 264B, and 326 at three inoculum concentrations for two generations on Bond oats (exp. 1)


First generation
Second generation
High concentration
Medium concentration
Low concentration
Number of pustules per leaf
0
40
80
120
160
200
240
280
X-421
C-649
216
X-421
C-649
264A
X-421
C-649
264B
X-421
C-649
326
Cultivar and race

(Table 25) and C-649 was attacked more heavily by race 264A.

Average ratios of resistant to susceptible infection types varied as a result of inoculum in experiment 2 (Table 9). Significant differences among generations, mixtures, and concentrations were obtained and the only interaction that did not show significance was generation x concentration (Table 10). The ratio in mixture 216+264A on cultivar X-421 varied inversely with concentration. The same mixture with C-649 presented the lowest ratio at the medium inoculum concentration and the general ratios were higher than those for cultivar X-421, and generally greater than one (Table 9). This means that the isolate of race 216 increased faster on C-649 than on X-421, and that there were more pustules of race 216 on C-649 than of 264A. Mixture 264A+264B had a direct response to inoculum concentration in the first generation on both cultivars, showing that race 264B decreased in the mixture with the decrease in concentration. The differences between generations showed that race 264B decreased in the mixture at the high concentration on X-421, but that it did not vary much with the other concentrations. The same race increased in the mixture on cultivar C-649 at all concentration levels. The difference between cultivars was significant (Table 25).

Ratios of the mixture of four races varied inversely with concentration when races 216, 264B, and 326 were compared with 264A on X-421, and those isolates decreased in the mixture in the second generation, with the greater difference at the medium and low levels (Table 9). The isolate of race 264A, in the same mixture, increased inversely with concentration on C-649 during the first generation, but in the second generation the highest ratio was for the medium concentration, decreasing significantly at the

Table 9. Average ratios of resistant to susceptible infection types (R/S) on X-421 and C-649 oats inoculated with six mixtures of crown rust races at three inoculum concentrations for two generations (exp. 2)

Concentration	Cultivar, R/S and generation					
	X-421			C-649		
	R/S	I	II	R/S	I	II
High	216/264A	0.26	0.36	216/264A	0.93	2.14
Medium		0.55	0.59		0.87	1.45
Low		0.85	0.68		1.05	1.89
High	-	-	-	216/264B	0.70	0.68
Medium		-	-		0.64	0.62
Low		-	-		0.70	1.16
High	-	-	-	216/326	1.03	0.80
Medium		-	-		0.68	0.80
Low		-	-		0.94	1.08
High	264B/264A	0.58	0.35	264A/264B	0.59	0.51
Medium		0.39	0.45		0.73	0.63
Low		0.28	0.29		1.14	0.51
High	326/264A	0.15	0.17	-	-	-
Medium		0.17	0.19		-	-
Low		0.10	0.44		-	-
High	216+264B + 326/264A	0.74	0.51	216/264A+264B + 326	0.55	0.83
Medium		0.91	0.58		0.82	0.88
Low		0.97	0.59		0.82	0.61

Table 10. Mean squares from the analysis of variance of the number of pustules on X-421 and C-649 oats inoculated with mixtures of crown rust races at three inoculum concentrations for two generations (exp. 2)

Source of variation	Cultivar	
	X-421	C-649
Generation	0.0754012 **	0.5872533 **
Mixture	0.8618160 **	1.4668250 **
G x M	0.1580642 **	0.8291233 **
Concentration	0.1131055 **	0.2685224 **
G x C	0.0017055	0.0269679
M x C	0.1064759 **	1.1045453 **

** F value exceeds 1% level of significance.

low level. The mixtures 216+264B and 216+326 on C-649 both showed a decrease in race 216 at the medium inoculum concentration, and higher but similar values at the other concentrations. This result is similar to that described for mixture 216+264A and it is important to keep in mind since the medium inoculum concentration was used in experiments 3 and 4.

The average spore germination percentage values (Table 11) varied between generations with higher values generally being obtained in the second generation. The spore germination test showed differences to be highly significant for generations, races, and their interaction in experiment 1 (Table 12). The high germination value for race 264A in the second generation in comparison to the other races appears to be responsible for the highly significant difference in the analysis of variance.

Effect of Temperature on Mixtures of Fungus Isolates

Experiments 3 and 4 were planned to study the influence of different temperature regimes on the survival of the four races used in mixtures in experiments 1 and 2. The temperatures selected for the day/night regimes in the three Percival growth chambers were taken from Taylor's (1967) study of the influence of temperature on the differentiation of oat genotypes. The regimes represent the average day/night temperatures during the oat growing season in Iowa. Taylor's (1967) calculations were made from data published by Shaw (1963). The three selected day/night temperature regimes (21/12, 26/17, and 32/19C) represent average temperatures during three periods between the last days in May and the first days in July. This also is the period during which _P. coronata_ builds up and causes damage in Iowa oat fields (Cournoyer, 1970).

Table 11. Average germination percentages of spores of four crown rust races at three inoculum concentrations used on the cultivar Bond for two generations (exp. 1)

Generation	Race	Inoculum concentration		
		Low	Medium	High
I	216	91	92	92
	264A	92	90	92
	264B	92	91	91
	326	91	91	91
II	216	90	93	91
	264A	98	97	97
	264B	94	92	93
	326	92	92	93

Table 12. Mean squares from the analyses of variance of the germination test in experiments 1 and 2

Source of variation	Experiment 1	Experiment 2
Generation	0.0080223 **	0.0001877
Race or mixture	0.0030074 **	0.0000361
Gen x R or M	0.0033851 **	0.0000683
Concentration	0.0000847	0.0005277
Gen x Conc.	0.0000847	0.0001077
Gen x R or M	0.0004587	0.0001069

** F value exceed 1% level of significance.

Table 13 shows the nine treatments of different combinations of three temperature regimes and four generations applied to Bond seedlings inoculated with the four races and six mixtures used in experiments 1 and 2. The temperature regimes prevailed inside the lamp chimneys. Laboratory thermometers in the lamp chimneys were checked and the growth chambers adjusted until the desired regime was obtained. An inoculum concentration of 10 mg of spores in 15 ml of Mobilsol 100 was used in this experiment. The combination of lamp chimneys plus the different temperature regimes created unique environments for the different treatments. Moisture was high at all times in the 21/12C Plant Growth Lab, but in the other two Plant Growth Labs, although it was high during darkness, moisture decreased during the day as temperatures increased. The increase in moisture decreased the light intensity that reached plants inside the lamp chimneys, especially those in the 21/12C regime.

The races and race mixtures differed significantly among generations for the average number of pustules/plant (Table 14). Mixtures gave poor infection in the fourth generation and mixtures 216+264B and 216+326 were eliminated before I checked the ratio of each isolate on C-649. Race 264A presented the lowest value in generation 2 with 27 pustules/plant; however, the same race had an average value of 80 and it was race 264B that decreased to 16 pustules/plant. The temperature effect was highly significant (Table 15) and the 32/19C regime, especially, caused a decrease in the number of pustules/plant. This difference was more obvious when conditions for infection were excellent as in the third generation for races 216 and 264A. The number of pustules/plant in treatment 9, that in the beginning was the lowest of all treatments, increased with generations as was especially clear

Table 13. Day/night temperature (C) regimes[a] in which the four crown rust races and six race mixtures used in experiments 3 and 4 developed for four uredial generations on seedling leaves of Bond oats

Treatment No.	Generation			
	I	II	III	IV
1	L	L	L	L
2	L	L	L	M
3	L	L	M	M
4	L	M	M	M
5	M	M	M	M
6	M	M	M	H
7	M	M	H	H
8	M	H	H	H
9	H	H	H	H

[a] L = 21/12 C, M = 26/17 C, H = 32/19 C.

Table 14. Average number of pustules per Bond oat seedling leaf inoculated with four crown rust races and six race mixtures and held under nine different temperature regimes for four generations (exp. 3 and 4).

		Race or Mixture									
Generation	Treatment	216	264A	264B	326	216+ 264A	216+ 264B	216+ 326	264A+ 264B	264A+ 326	216+264A+ 264B+326
I	1	37	40	63	50	74	40	62	75	46	56
	2	45	35	65	54	65	46	58	79	40	51
	3	38	40	61	45	74	42	55	65	42	50
	4	39	39	55	48	67	48	58	68	47	57
	5	42	39	51	43	62	43	54	71	43	67
	6	41	35	50	38	56	46	70	68	44	69
	7	46	38	44	34	58	51	67	65	54	71
	8	43	33	45	38	55	47	62	66	49	65
	9	25	15	24	24	27	28	24	25	21	31
II	1	124	26	97	72	73	77	84	104	90	53
	2	105	35	65	71	99	71	76	72	82	45
	3	89	37	89	65	76	69	69	72	68	47
	4	125	27	86	58	85	56	84	69	85	45
	5	116	28	77	61	84	81	86	85	75	50
	6	111	26	83	72	66	74	70	84	74	66
	7	75	22	70	59	75	63	71	66	70	44
	8	44	28	50	24	16	30	23	18	24	32
	9	24	15	31	18	18	16	16	8	23	16
III	1	124	101	17	54	78	92	39	40	40	50
	2	126	110	13	40	63	71	32	35	43	37
	3	108	119	17	57	74	91	38	33	42	52
	4	145	145	17	90	121	55	37	34	43	44
	5	116	80	16	52	124	85	49	38	41	32
	6	114	76	18	51	76	86	31	36	39	45
	7	59	43	14	51	73	98	32	30	37	37
	8	53	21	20	33	49	73	22	25	33	42
	9	36	21	16	34	45	41	37	22	33	36
IV	1	39	19	69	21	20	-	-	10	18	34
	2	52	20	56	28	21	-	-	5	17	20
	3	49	46	30	18	13	-	-	9	15	19
	4	58	47	27	19	15	-	-	6	23	18
	5	58	32	38	22	16	-	-	9	16	12
	6	26	31	31	9	21	-	-	6	14	16
	7	21	24	31	8	13	-	-	7	9	9
	8	34	25	22	26	17	-	-	8	11	17
	9	44	34	25	41	11	-	-	3	18	3

for races 216, 264A, and 326. The inconsistency in quantitative values for the four races according to the different generations did not justify any further analysis of the data. Variation among the mixtures with generations was relatively small and the seedlings produced inoculum sufficient for the analysis of R/S ratios on index cultivars X-421 and C-649. The analysis of variance (Table 15) showed that the differences among races or mixtures and the effect of temperature were highly significant. The race or mixture x temperature mean squares also were highly significant.

The average number of pustules/plant on X-421 and C-649 inoculated with race 264A are presented in Table 16 and the analysis of variance is in Table 17. Highly significant differences were found for each cultivar for generations, races, temperature, and for the interactions of these factors. The general tendency in relation to the treatment was for the production of lower numbers of pustules on plants kept at the high temperature regime. Differences among generations were observed in both cultivars. X-421 and C-649 bore three times more pustules in the second generation than in any other. The number of pustules on X-421 and C-649 was similar for any generation; however, the difference in mean values 40.2694 (X-421) and 42.9491 (C-640) was significant when analyzed through the standard error of values (Table 25).

Table 18 presents the average germination percentages for experiment 3. All sources of variation differed significantly for this character (Table 19). Race 326 appeared to have the highest germination percentage over all treatments, and the spores of races 216 and 264A seemed only slightly affected by high temperature.

The results of experiment 4 are summarized in Tables 20-23 and

Table 15. Mean squares from the analysis of variance of the number of pustules of four crown rust races and six race mixtures on seedling leaves of Bond oats (exp. 3 and 4)

Source	Generation		
	I	II	III
Race or Mixture	380.624 **	12581.400 **	3771.014 **
Temperature	706.360 **	3648.841 **	256.650 **
R or M x T	20.813 **	699.533 **	137.159 **

** F value exceeds 1% level of significance.

Table 16. Average number of pustules/plant of crown rust race 264A indexed on X-421 and C-649 oat seedling leaves. Race 264A developed on Bond oats under different temperature regimes for four generations (exp. 3)

Treat-ment	Generation									
	X-421					C-649				
	I	II	III	IV	Gen. Mean	I	II	III	IV	Gen. Mean
1	31	94	17	11	38	41	89	20	23	43
2	29	101	15	27	43	37	105	18	29	47
3	30	114	20	35	50	38	120	21	45	56
4	31	100	28	28	47	37	116	29	54	59
5	38	68	31	42	45	34	65	37	45	45
6	33	73	21	39	42	34	73	22	46	44
7	34	76	12	28	38	32	79	11	39	40
8	30	49	18	33	32	29	50	19	39	34
9	18	49	19	28	28	18	17	17	18	18

Table 17. Mean squares from the analysis of variance of the number of pustules of four crown rust races and six race mixtures indexed on seedling leaves of X-421 and C-649 oats (exp. 3)

Source of variation	X-421	C-649
Generation	3897.868 **	9500.426 **
Race	7159.594 **	9864.531 **
G x R	3356.653 **	5299.426 **
Temperature	244.714 **	2943.454 **
G x T	157.031 **	563.660 **

** F value exceeds 1% level of significance.

Table 18. Average germination percentages (four generations) of spores of four crown rust races according to the treatment under which the inoculated Bond oat plants developed (exp. 3)

Treatment	Crown rust race			
	216	264A	264B	326
1	93	91	92	95
2	92	93	92	94
3	92	93	92	94
4	92	92	93	94
5	92	91	92	94
6	92	88	91	91
7	91	88	91	95
8	89	88	92	93
9	88	90	91	92

Table 19. Mean square from the analysis of variance of the spore germination tests in experiments 3 and 4

Source of variation	Experiment 3	Experiment 4
Generation	0.0033953 **	0.0002646
Race or Mixture	0.0129731 **	0.0088388
G x R or M	0.0036111 **	0.0048308
Temperature	0.0039445 **	0.0022552
G x T	0.0005003 **	0.0016448
R or M x T	0.0012010 **	0.0005931

** F value exceeds 1% level of significance.

Fig. 8-13. The analysis of variance of this experiment (Table 24) indicates that all sources of variation were highly significant. Table 20 contains data from the mixtures based on my isolate of race 216. The original data, recorded as the ratio of resistant to susceptible infection types, give a coefficient that always is related to one. For instances, if one obtains 10 resistant-type pustules and 20 susceptible ones, the coefficient 0.50 means we had a proportion of 0.5 to 1.0. The original coefficients were multiplied by 100 to obtain the value presented in Table 20. This enabled me to calculate the half-life values, in the concept of Van der Plank (1968), of one isolate as a function of the other(s) in a mixture. The method for calculating these values is explained clearly by Van der Plank (1968). It consists basically of keeping a proportion between the race that is decreasing in the mixture and the other race(s) so that the latter always equals 100, just as in Tables 20 and 22. The half-life value is found by using a logarithmic transformation for the values found for the decreasing race (called Y values) and obtaining a linear regression coefficient b value. The b value divided by the logarithm of 1/2 (-0.301) yields the half-life value of the decreasing race. Van der Plank's method works exactly the same whether one wants to calculate "half-life" or "half-increase" since both data are dependent and the regression coefficient b value indicates the half-life when it is negative and the half-increase when it is positive (Tables 21 and 23, Fig. 8-13).

The values in Table 20 show that race 216 increased throughout the four generations in all mixtures except in the one with race 326, and this was true for all treatments or temperature regimes studied. The half-life values (Table 21 and Fig. 8-10) indicate that one half of the race 264A

Table 20. Number of pustules[a] of crown rust race 216 indexed on seedling leaves of X-421 and C-649 oats inoculated with two- or four-race mixtures that had developed under nine different temperature regimes for four generations on Bond oats (exp. 4)

Treatment	Generation	Index Cultivar and Mixture				
		X-421	C-649	C-649	C-649	C-649
		216+264A	216+264A	216+264B	216+326	216+264A+264B +326
1	I	48	145	74	116	68
	II	62	157	95	97	71
	III	60	303	310	91	100
	IV	147	297	-	-	-
2	I	36	132	76	113	69
	II	61	138	96	76	80
	III	72	238	316	75	111
	IV	293	376	-	-	139
3	I	53	156	74	118	75
	II	64	142	96	99	82
	III	41	337	297	99	121
	IV	666	521	-	-	136
4	I	50	129	74	115	73
	II	67	183	93	89	101
	III	70	346	304	94	92
	IV	402	271	-	-	134
5	I	79	120	66	105	121
	II	99	135	91	79	115
	III	61	232	242	71	83
	IV	256	355	-	-	182
6	I	81	119	65	100	95
	II	98	143	75	92	94
	III	80	343	235	84	83
	IV	537	317	-	-	129
7	I	94	159	64	104	89
	II	88	144	78	87	93
	III	69	351	221	77	94
	IV	425	463	-	-	165
8	I	91	130	63	101	78
	II	88	154	62	72	82
	III	71	357	233	58	84
	IV	309	587	-	-	108
9	I	46	82	41	103	50
	II	57	139	89	109	107
	III	65	236	208	71	115
	IV	153	244	-	-	108

---

[a] Number of pustules given is that for race 216 (underlined) relative to a fixed value of 100 for the other race(s) in each mixture.

Table 21. Half-life and regression-coefficient values[a] for crown rust races 216, 264A, 264B, and mixture 264A+264B+326 according to different index cultivars and treatments (exp. 4)

		Race 216 in mixture with							
Treatment	Cultivar	264A		264B		326		264A+264B+326	
		b value	half-life	b value	half-life	b value	half-life	b value	half-life
1	X-421	0.15	2.00	-	-	-	-	-	-
	C-649	0.12	2.51	0.31	0.97	-0.05	6.02	0.14	2.15
2	X-421	0.32	0.94	-	-	-	-	-	-
	C-649	0.17	1.77	0.31	0.97	-0.09	3.34	0.11	2.75
3	X-421	0.32	0.94	-	-	-	-	-	-
	C-649	0.20	1.50	0.30	1.00	-0.04	7.53	0.09	3.36
4	X-421	0.27	1.12	-	-	-	-	-	-
	C-649	0.14	2.51	0.35	0.86	-0.05	6.02	0.08	3.76
5	X-421	0.13	2.32	-	-	-	-	-	-
	C-649	0.17	1.77	0.29	1.03	-0.09	3.34	0.04	7.50
6	X-421	0.24	1.26	-	-	-	-	-	-
	C-649	0.16	1.88	0.28	1.08	-0.04	7.53	0.03	10.00
7	X-421	0.19	1.59	-	-	-	-	-	-
	C-649	0.18	1.68	0.27	1.11	-0.07	4.30	0.08	3.76
8	X-421	0.15	2.00	-	-	-	-	-	
	C-649	0.25	1.20	0.29	1.04	-0.12	2.51	0.08	6.00
9	X-421	0.16	1.88	-	-	-	-	-	-
	C-649	0.17	1.77	0.36	0.84	-0.08	376	0.12	2.52

[a] Negative b values indicate the half-life of race 216, while positive values indicate the half-life for the other race(s) in mixtures with race 216.

Table 22. Number of resistant-type crown rust pustules[a] indexed on seedling leaves of X-421 and C-649 oats inoculated with race 264A in two- or four-race mixtures that had developed under nine different temperature regimes for four generations on Bond oats (exp. 4)

Treatment	Generation	Cultivar and Mixture			
		X-421	C-649	X-421	X-421
		264A+264B	264A+264B	264A+326	264A+216+264B +326
1	I	89	64	9	125
	II	64	56	16	108
	III	37	65	17	582
	IV	70	92	-	644
2	I	85	72	9	118
	II	56	71	19	98
	III	58	112	19	397
	IV	52	138	-	443
3	I	91	73	7	119
	II	57	76	15	99
	III	12	83	15	366
	IV	33	123	-	403
4	I	105	58	7	119
	II	56	72	15	93
	III	20	88	22	325
	IV	47	117	-	438
5	I	79	68	8	69
	II	52	71	15	73
	III	50	111	16	138
	IV	46	126	-	137
6	I	81	74	7	58
	II	47	85	18	58
	III	12	88	20	77
	IV	63	94	-	105
7	I	73	72	8	69
	II	51	70	15	81
	III	32	85	24	93
	IV	48	106	-	126
8	I	75	77	8	71
	II	71	115	19	148
	III	40	116	21	145
	IV	45	117	-	174
9	I	77	83	12	149
	II	44	66	24	150
	III	35	159	32	152
	IV	46	115	-	170

[a] Number of pustules is that for the race(s) underlined relative to a fixed value of 100 for the other race in the mixture.

Table 23. Half-life and regression-coefficient values for crown rust races 264A and 264B, according to different index cultivars and treatments (exp. 4)

Treatment	Cultivar	Race 264A in mixture with					
		264B[a]		326[b]		216+264B+326[b]	
		b value	half-life	b value	half-life	b value	half-life
1	X-421	0.05	6.02	-0.14	2.15	-0.29	1.05
	C-649	0.05	6.02	-	-	-	-
2	X-421	0.06	5.00	-0.11	2.14	-0.24	1.26
	C-649	0.10	3.01	-	-	-	-
3	X-421	0.20	1.50	-0.11	2.14	-0.18	1.68
	C-649	0.07	4.30	-	-	-	-
4	X-421	0.14	2.15	-0.25	1.20	-0.22	1.38
	C-649	0.10	3.01	-	-	-	-
5	X-421	0.07	4.20	-0.15	2.00	-0.12	2.52
	C-649	0.10	3.01	-	-	-	-
6	X-421	0.09	2.36	-0.23	1.31	-0.09	3.33
	C-649	0.03	10.0	-	-	-	-
7	X-421	0.07	4.30	-0.24	1.25	-0.08	3.76
	C-649	0.06	5.00	-	-	-	-
8	X-421	0.13	2.21	-0.21	1.43	-0.12	2.51
	C-649	0.06	5.00	-	-	-	-
9	X-421	0.08	3.76	-0.22	1.37	-0.02	15.00
	C-649	0.08	3.76	-	-	-	-

[a] Values for half-life of race 264B.

[b] Values for half-life of race 264A.

Table 24. Mean squares from the analysis of variance of the number of pustules on index oat cultivars X-421 and C-649 (exp. 4)

Source of variation	X-421	C-649
Generation	30.304 **	16.327 **
Race	33.243 **	51.449 **
G x R	15.010 **	6.194 **
Temperature	2.046 **	0.519 **
G x T	1.284 **	0.267 **
R x T	3.276 **	0.562 **

** F value exceeds 1% level of significance.

Figs. 8-10. Increase per generation on Bond oats growing under 21/12, 26/17, and 32/19 C day/night temperature regimes of crown rust race 216 in a mixture with race 264A, and half-life of race 264A in a mixture with race 216, indexed on X-421 and C-649 oats

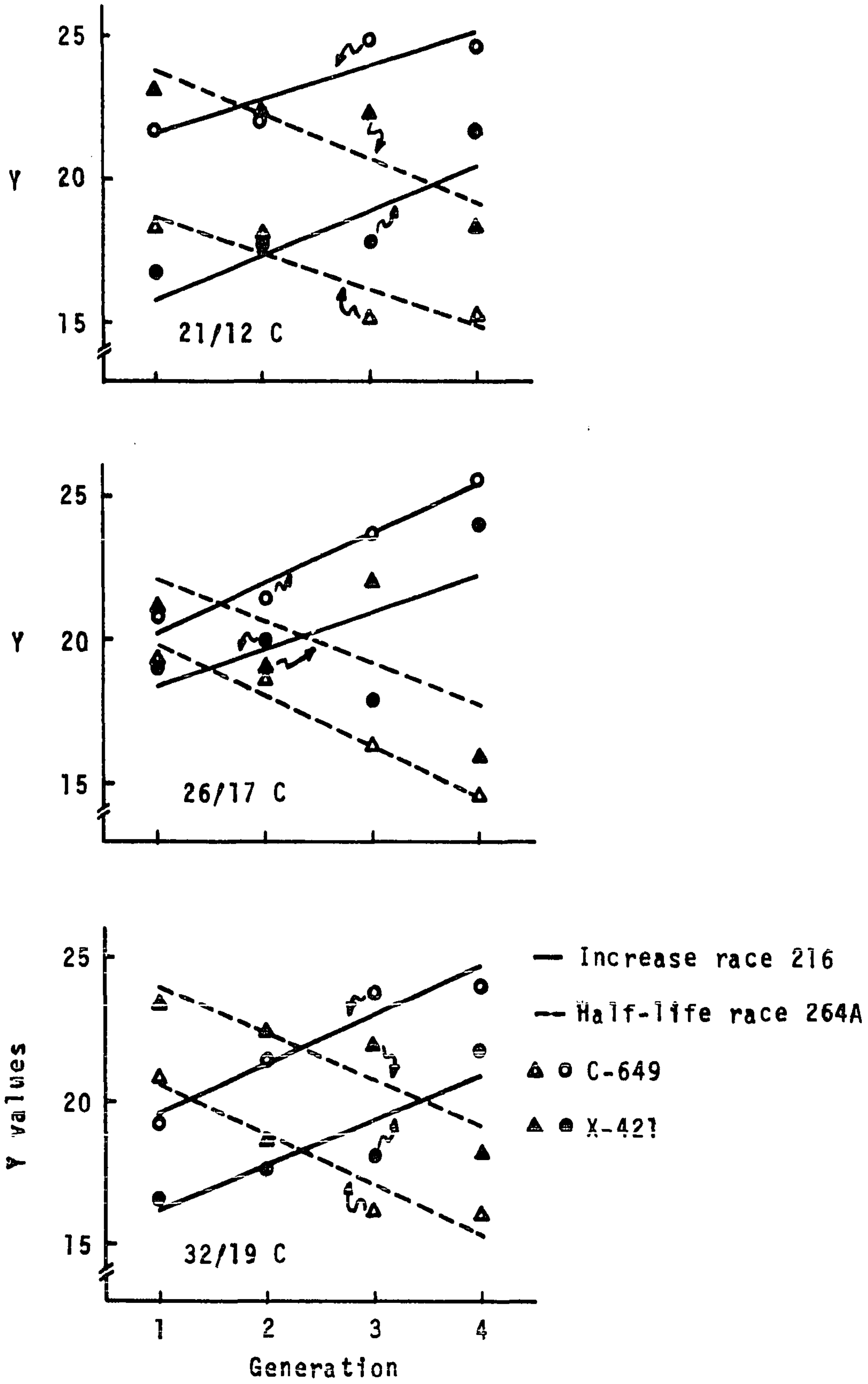
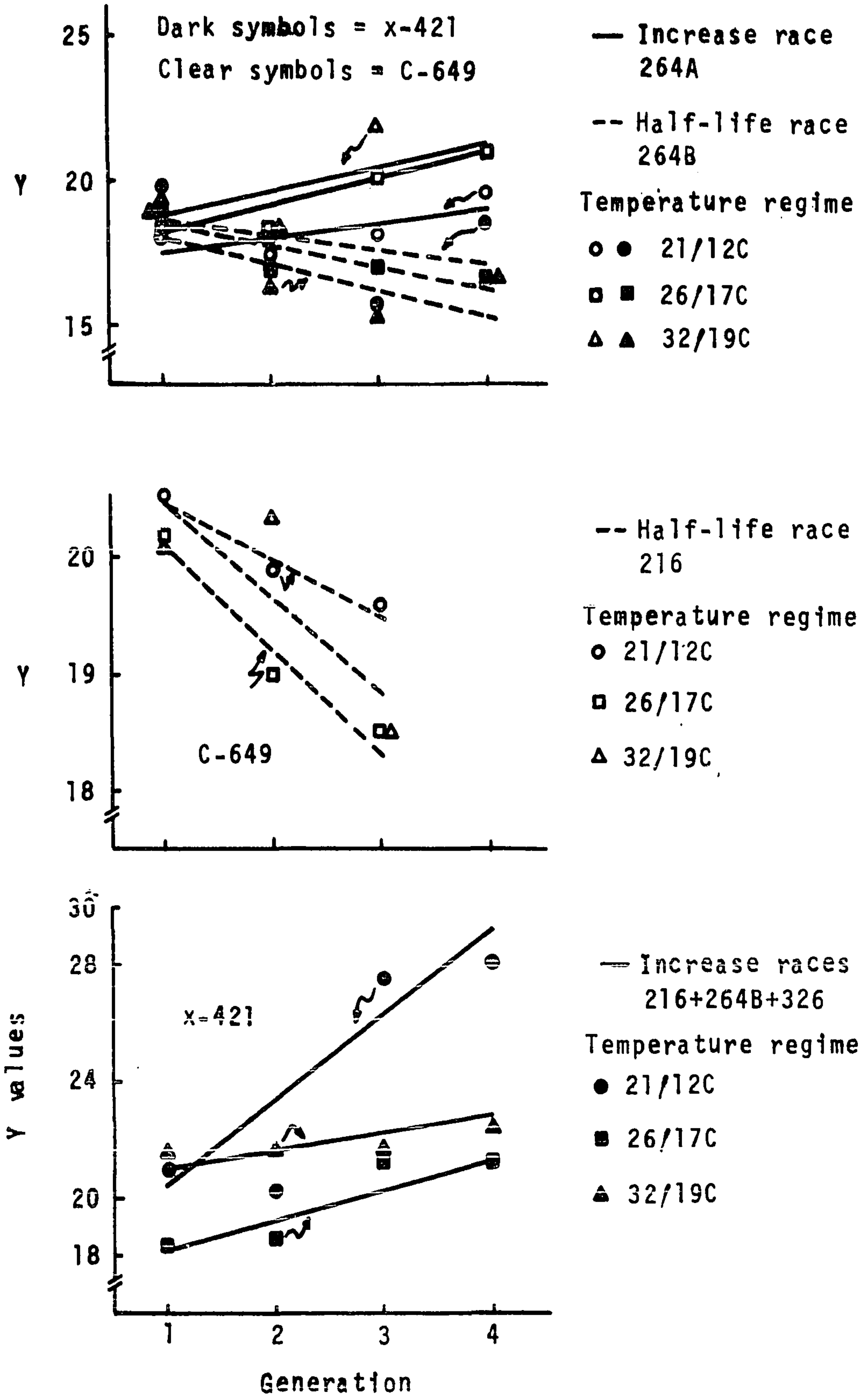

25
Y 20
15
21/12 C
25
Y 20
15
26/17 C
25
Y values 20
15
32/19 C
1
2
3
4
Generation
— Increase race 216
-- Half-life race 264A
△ ○ C-649
▲ ● X-421

Fig. 11. Increase per generation on Bond oats growing under 21/12, 26/17, and 32/19 C day/night temperature regimes of crown rust race 264A in mixture with race 264B indexed on X-421 oats, and half-life of race 264B in a mixture with race 264A indexed on C-649 oats

Fig. 12. Half-life of crown rust race 216 in a 216+326 mixture that developed on Bond oats for four generations at 21/12, 26/17 and 32/19 C. Indexing was on C-649 oats

Fig. 13. Increase of crown rust races 216, 264B, and 326 in a mixture with race 264A. The mixture developed on Bond oats for four generations at 21/12, 26/17, and 32/19 C. Indexing was on X-421


Dark symbols = x-421
Clear symbols = C-649
— Increase race 264A
-- Half-life race 264B
Temperature regime
○ ● 21/12C
□ ■ 26/17C
△ ▲ 32/19C
Y
25
20
15
-- Half-life race 216
Temperature regime
○ 21/12C
□ 26/17C
△ 32/19C
C-649
Y
20
19
18
— Increase races 216+264B+326
Temperature regime
● 21/12C
■ 26/17C
▲ 32/19C
x-421
Y values
30
28
24
20
18
1
2
3
4
Generation

population dropped from a mixture with race 216 in a period of time between 0.94 and 2.5 generations, depending on the temperature regime but without having a particular trend and being similar on the index cultivars X-421 and C-649. Race 264B presented half-life values of close to one generation in the mixture with race 216 when indexed on C-649. The race 216+326 mixture showed that race 216 decreased in the mixture and its half-life values were from 2.51 to 7.53 generations (Fig. 12). The mixture of race 216 and the other three races gave half-life values of from 2.15 to 10.00 generations for the three races in the mixture with race 216.

Table 23 presents data for the half-life of race 264B in a mixture with race 264A (Fig. 11) and also the values for race 264A in mixtures with 326 alone or with 216+264B+326 (Fig. 13). These data indicate that race 264B decreased in a 264A+264B population, but that 264A decreased in the presence of race 326 or in a mixture with the other three races. The half-life values for race 264A in the mixture with 216 are similar to those found with 326 when indexed on X-421. The mean values in Table 25 indicate that the ratio of resistant to susceptible infection types is significantly different between index cultivars X-421 and C-649 inoculated with mixtures 216+264A, 264A+264B, or 216+264A+264B+326.

Mean squares from the analysis of variance (Table 19) for the germination test in experiment 4 did not indicate any significant difference in this character; however, the germ tube length, evaluated using spores from the fourth generation of experiments 3 and 4 four and 24 hr after the germination test started, indicated clear differences in the pattern of germ tube development (Tables 26 and 27). The spores of race 216 are the ones that germinated faster and produced longer germ tubes at any temperature

Table 25. Means and standard-error values of the number of pustules on index oat cultivars X-421 and C-649. Inoculum was of crown rust race 264A or three different race mixtures that developed on Bond subjected to three concentrations (exp. 1 and 2) or three temperature regimes (exp. 3 and 4)

Cultivar	Race or Mixture	Experiment No.	Mean Value		Standard Error
X-421	264A	1	78.0761	±	2.3382
C-649	264A	1	104.2760	±	2.5299
X-421	264A+216	2	0.5478	±	0.0142
C-649	264A+216	2	1.3894	±	0.0732
X-421	264A+264B	2	0.3872	±	0.0142
C-649	264A+264B	2	0.6872	±	0.0732
X-421	216+264A+264B+326	2	0.7117	±	0.0142
C-649	216+264A+264B+326	2	0.7572	±	0.0732
X-421	264A	3	40.2694	±	0.4206
C-649	264A	3	42.9491	±	0.7696
X-421	264A+216	4	1.4256	±	0.0278
C-649	264A+216	4	2.4082	±	0.0316
X-421	264A+264B	4	0.5808	±	0.0278
C-649	264A+264B	4	0.8832	±	0.0316
X-421	216+264A+264B+326	4	1.9251	±	0.0728
C-649	216+264A+264B+326	4	1.0136	±	0.0316

Table 26. Average length of the germ tubes from spores of crown rust races 216, 264A, 264B, and 326 after 4 and 24 hr of germination. Spores were from the fourth generation on Bond and developed under nine treatments (exp. 3)

Race	Germination time (hr)	Treatment								
		1	2	3	4	5	6	7	8	9
216	4	10	11	14	11	15	10	9	12	12
	24	16	14	22	16	15	10	9	12	12
264A	4	4	6	6	10	4	4	8	5	10
	24	10	15	23	14	10	4	8	5	10
264B	4	8	4	2	5	3	3	7	6	8
	24	8	7	5	10	6	3	10	8	10
326	4	8	16	11	8	13	16	10	8	8
	24	8	18	26	10	17	16	14	17	9

Table 27. Average length of the germ tubes from the spores of six crown rust race mixtures after 4 and 24 hr of germination. Spores were from the fourth generation on Bond and developed under nine treatments (exp. 4)

Mixture	Germination time (hr)	Treatment								
		1	2	3	4	5	6	7	8	9
216+264A	4	9	10	12	12	15	19	6	11	5
	24	9	12	13	14	15	19	10	16	6
264A+264B	4	4	4	3	2	3	2	1	4	3
	24	4	4	5	2	3	2	2	4	4
264A+326	4	5	3	3	10	7	9	10	2	2
	24	6	11	8	10	8	9	10	3	4
216+264A	4	4	4	6	10	8	4	2	5	8
	24	12	5	23	10	8	4	4	6	8

regime after 4 and 24 hr. The germ tubes of race 326 also grew fast, but races 264A and 264B presented the least germ tube development after 4 and 24 hr. These results suggested the study of the interaction of oil, temperature, and moisture on the germination of *P. coronata* uredospores.

Leaf-Wetness Duration, Infection, and Spore Production

In order to supplement and possibly explain the results of the inoculum concentration and temperature experiments, races 216, 264A, 264B, and 326 were studied in relation to 1) duration of moisture required to effect infection and 2) spore production. These experiments included the effect of the duration of leaf wetness during germination and penetration, the period between inoculation and the eruption of uredia, duration of uredospore production, and onset and intensity of telial formation.

Leaf-wetness duration and the resultant infection The effect of the time in a moist chamber with leaves wet was studied using the cultivar Bond. The seedlings were inoculated, wet with distilled water, and moved to a moist chamber in an air conditioned 21 ± 1C lab. The material was divided into three groups that were removed after 4.5, 5.5, or 14.0 hr, respectively, in the moist chamber. The plants were dried immediately. One experiment was conducted in the greenhouse with a temperature of 21 ± 2C and 70-85% relative humidity; the other experiment was run in a Plant Growth Lab with a 21/17C day/night temperature regime and 40-50% relative humidity. Seedlings were not covered with lamp chimneys and data on the number of pustules/leaf were analyzed using the log X+1 transformation since the pustule number was low at the 4.5-hr treatment and some plants did not show any signs of infection. Table 28 and Fig. 14 indicate that plants kept in the greenhouse produced more pustules than those in the Plant Growth Lab, but since they corresponded to different inoculations it is not possible to infer any correlation between these two experiments. Fig. 14 shows that race 264A produced the fewest pustules in both experiments in the 4.5-hr treatment, but that it went to higher levels in the 5.5- and 14.0-hr treatments. Races 264B and 326 were highly affected when the 4.5-hr treatment was followed by low relative humidity conditions (Plant Growth Lab) compared to race 216. Race 216 presented a linear increase in the Plant Growth Lab and it gave almost the same response in the greenhouse. It is interesting to observe that the number of pustules in the 14.0-hr treatment was very similar in both experiments for races 264A and 264B, but that it was quite different for races 216 and 326.

Period between inoculation and the eruption of uredia The values in Table 29 represent the average period required for eruption of uredia over four generations. The period was at least a day shorter in the material covered with lamp chimneys in comparison with that without lamp chimneys (Table 29). All isolates developed faster under the 26/17 or 24/24C temperature regimes than under the 21/12 or 32/19C regimes. Races 216 and 326 had shorter periods between inoculation and the eruption of uredia than races 264A and 264B at any temperature.

Sporulating area and duration of spore production The sporulating area of the uredia of races 216, 264A, 264B, and 326 on Bond was measured from photographs taken 9 and 14 days after inoculation (Fig. 15-22). A centimeter scale in each picture was the reference point for magnification. The area measurements, made with a planimeter "Salmoiraghi,"[1/] are

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[1/] Model No. 236/A manufactured by Fitotecnia Salmoiraghi S.P.A. Milano 5 VIA R SANZIO, Italy.

Fig. 14. Relationship between duration of leaf wetness and number of pustules of four crown rust races that developed on Bond oats

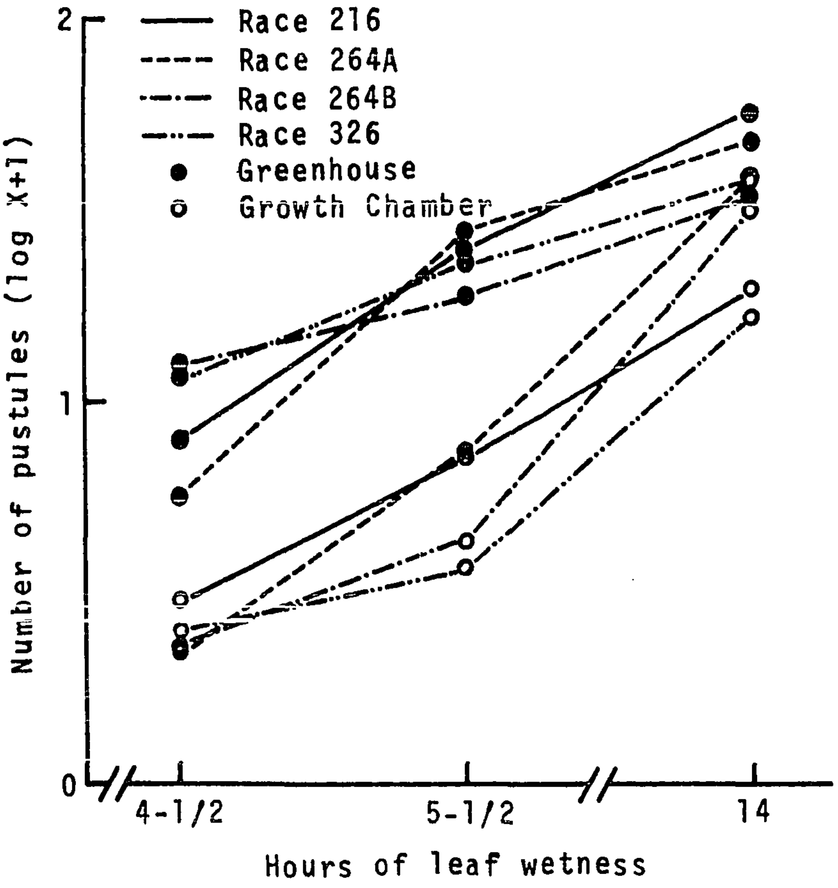

2
1
0
Race 216
Race 264A
Race 264B
Race 326
Greenhouse
Growth Chamber
Number of pustules (log X+1)
4-1/2
5-1/2
14
Hours of leaf wetness

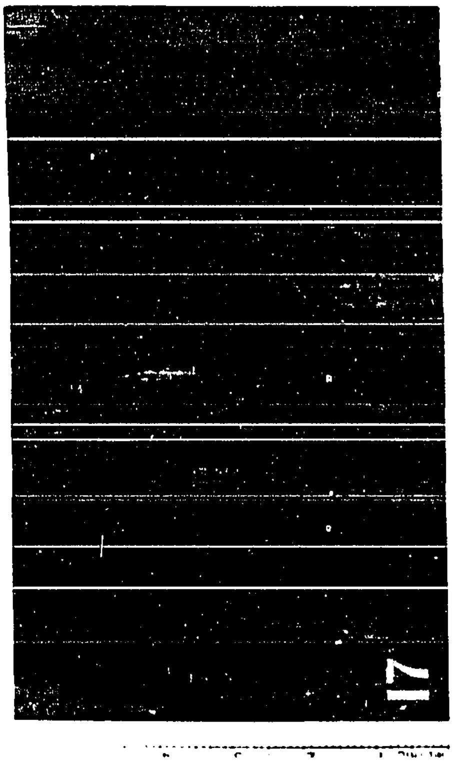
Table 28. Average number of pustules of crown rust races 216, 264A, 264B, and 326 that developed on Bond oats that were inoculated and kept 4.5, 5.5, and 14.0 hr in a moist chamber and then held under greenhouse and growth chamber conditions during the infection period

Place	Race	Duration of leaf wetness (hr)		
		4.5	5.5	14.0
Greenhouse	216	7.78	25.13	56.24
	264A	5.76	26.91	47.90
	264B	12.60	18.20	33.90
	326	12.03	24.00	38.02
Growth Chamber	216	2.00	5.91	23.05
	264A	1.18	6.25	32.08
	264B	1.25	3.35	32.40
	326	1.54	2.55	16.21

Table 29. Average period in days between inoculation and the eruption of uredia of crown rust races 216, 264A, 264B, and 326 that developed on Bond oats under four different temperature regimes

Temperature regime day/night (C)	216	264A	264B	326
Lamp chimney				
21/12	9	10	9	8
26/17	7	8	7	6 1/2
24/24	7	8	7	6 1/2
32/19	8	9	9	8
No lamp chimney				
26/17	8	9	9	8

Figs. 15-18. Isolates of crown rust races 216, 264A, 264B, and 326 inoculated onto Bond oat seedlings and exposed from left to right to 21/12, 26/17, 24/24, and 32/19 C temperature regimes. Photographed nine days after inoculation



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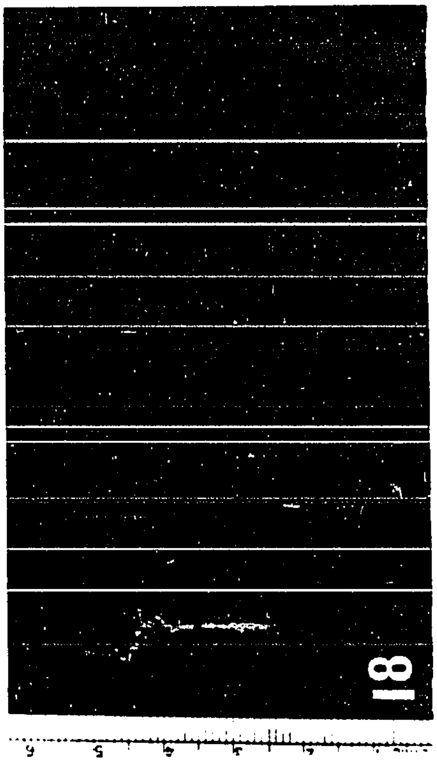

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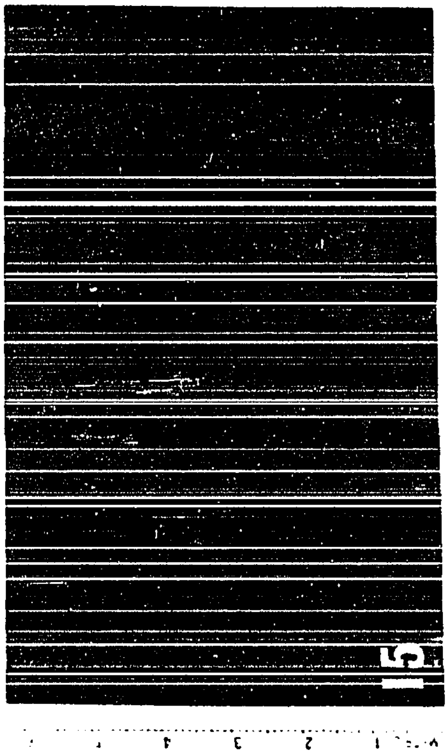

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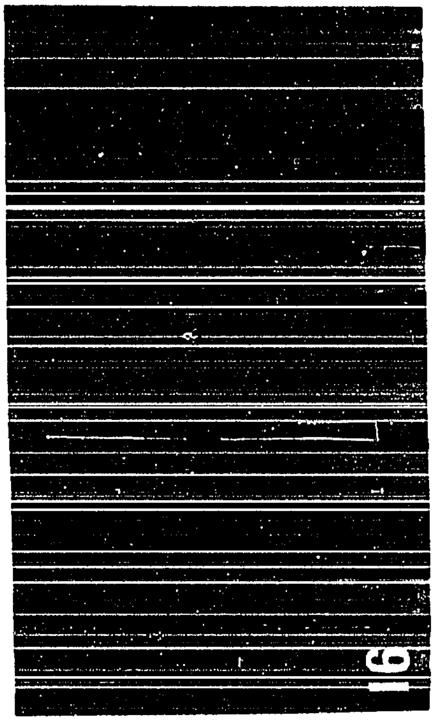
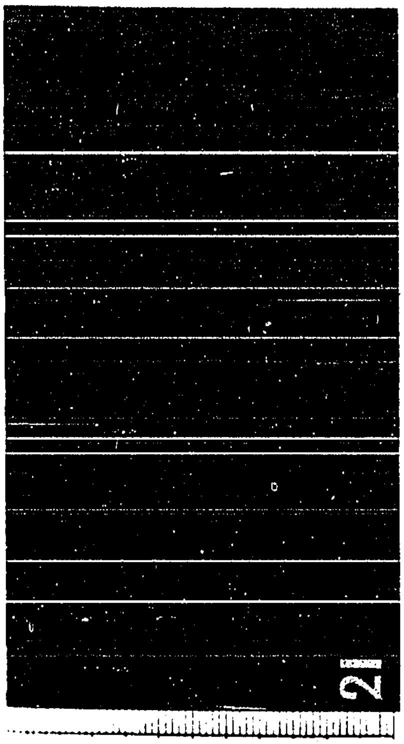


16

Figs. 19-22. Isolates of crown rust races 216, 264A, 264B, and 326 inoculated onto Bond oat seedlings and exposed from left to right to 21/12, 27/17, 24/24, and 32/19 C temperature regimes. Photographed 14 days after inoculation



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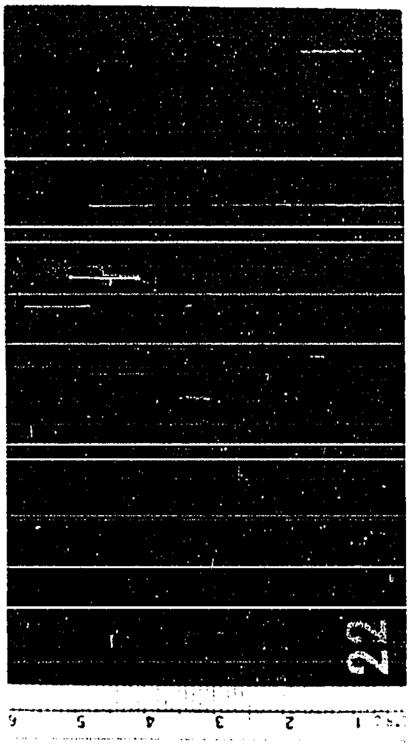

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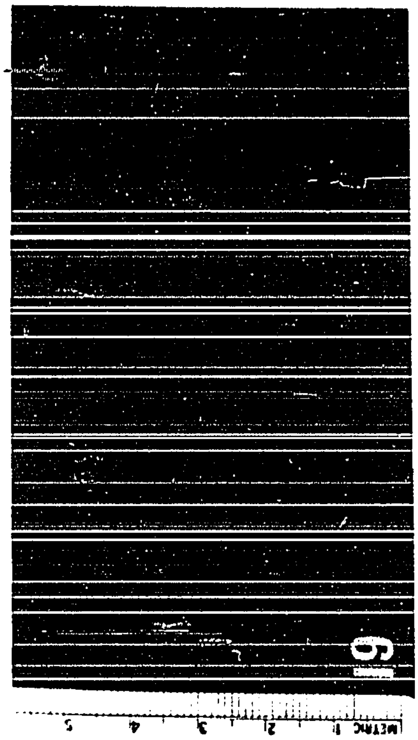

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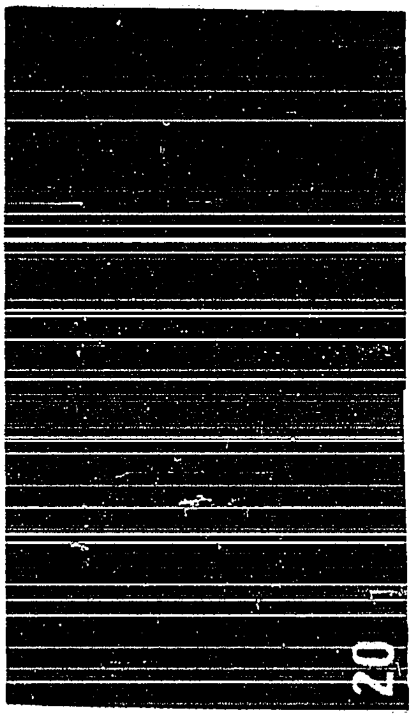

20

presented in Table 30 and Fig. 23. Race 326 had the largest sporulating area, at any temperature regime, nine days after inoculation. Also, all races presented small sporulating areas at 21/12C and 32/19C temperature regimes. This may be due to the longer generation times at these temperature regimes. Race 326 had the biggest sporulating area at 26/17 and 24/24C 14 days after inoculation; however, the area decreased significantly at the 21/12 and 32/19C temperature regimes (Fig. 23). Race 264B kept high values for sporulating area and did not present significant decrease due to high or low temperatures. Uredia of race 264A had the smallest area of the four races and it was quite similar for all temperature regimes. Race 216 presented a variable pattern of sporulating area similar to that of race 326, according to the temperature regime; however, the area size was small and close to that of race 264A.

All races produced spores actively for ca. 25 days at the 21/12C regime and 21 days at the other regimes.

Telial formation The presence or absence of telial formation was evaluated on Bond, X-421, C-649, and the crown rust standard differential cultivars (Table 31). Observations were made as long as 28 days after inoculation. Race 326 produced telia on Appler, Bond, Bondvic, and Trispernia. Race 326 formed telia only as part of secondary sporulation on a given suscept around 21 days after inoculation and the primary pustules continued to produce uredospores. Race 216 developed telia on Appler, Landhafer, X-421, and C-649. X-421 was the only cultivar in which race 264B produced telia. Race 264A did not include telial formation on any of the cultivars tested during the time I made observations.

Table 30. Sporulating area (10^{-2} mm^2) of uredia of crown rust races 216, 264A, 264B, and 326 that developed on Bond oats kept at four different temperature regimes for 9 and 14 days after inoculation

Race	Days after inoc.	Day/night temperature (C)			
		21/12	26/17	24/24	32/19
216	9	21	32	23	15
	14	28	40	46	32
264A	9	17	24	22	12
	14	26	35	34	31
264B	9	21	31	37	12
	14	47	48	55	52
326	9	26	44	33	28
	14	41	57	60	42

Fig. 23. Sporulating area (in 10^{-2} mm^2) of uredia produced by four crown rust races that developed on Bond oats kept at four different temperature regimes for 9 and 14 days after inoculation

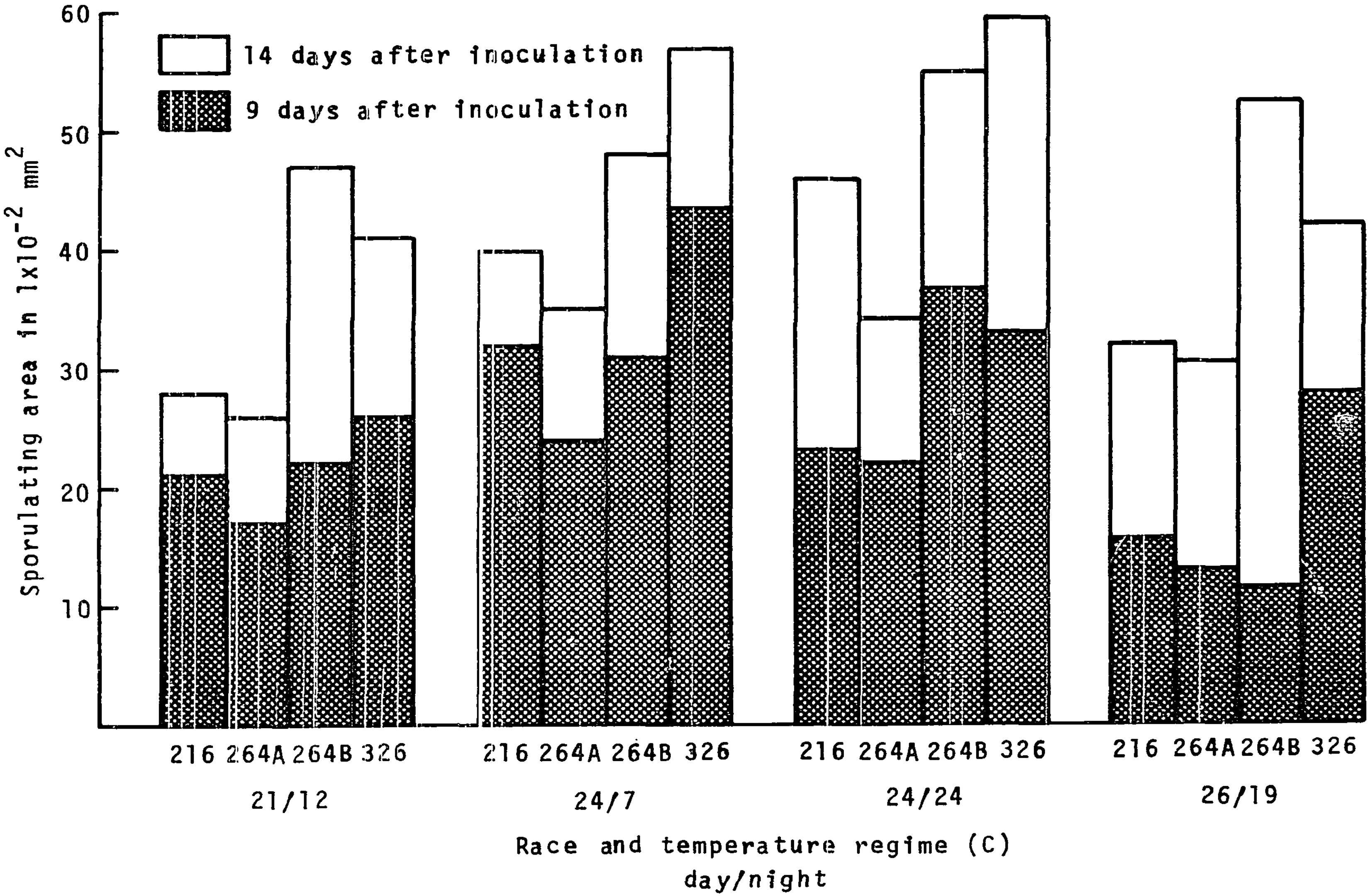
14 days after inoculation
9 days after inoculation
Sporulating area in 1×10^{-2} mm^2
60
50
40
30
20
10
216 264A 264B 326
21/12
216 264A 264B 326
24/7
216 264A 264B 326
24/24
216 264A 264B 326
26/19
Race and temperature regime (C)
day/night

Table 31. Presence (+) or absence (-) of telial formation by crown rust races 216, 264A, 264B, and 326 on seven oat cultivars

	Cultivar						
Race	Appler	Bond	Bondvic	Landhafer	Trispernia	X-421	C-649
216	+	-	-	+	-	+	+
264A	-	-	-	-	-	-	-
264B	-	-	-	-	-	+	-
326	+	+	+	-	+	-	-

Table 32. Average germination percentages of spores of crown rust races 216, 264A, 264B, and 326 collected and stored 48 hr in a desiccator and then rehydrated in a moist chamber for 0, 3, 12, or 24 hr prior to germination on water agar

	Time in	Hours rehydration			
Race	oil	0	3	12	24
216	0	30	20	25	-
	1 min	97	96	94	95
	4 hr	96	0	0	0
264A	0	60	50	50	-
	1 min	97	96	96	95
	4 hr	97	0	0	0
264B	0	60	70	70	60
	1 min	98	97	97	97
	4 hr	97	0	0	0
326	0	60	60	60	60
	1 min	98	97	97	96
	4 hr	97	0	0	0

Effect of Oil and Moisture on Germination

Germ tube development in the spore germination tests in experiments 3 and 4 (Table 26 and 27) indicated that some factor(s) was interfering with the normal germination process in some isolates, and possibly, giving some selective advantage to others.

Temperature, as a factor possibly affecting germination, was studied at two levels, 5 and 20C. I found no difference among races, and the only difference between temperatures was a slower rate of germination at 5C.

Next I tested the effect of hydration on spores kept in a desiccator for 48 hr, just as in the different experiments, and studied results of the interaction between oil and spores after the rehydration process.

Oil did not adversely affect germination on non-hydrated spores; in fact, one min or four hr in oil enhanced their germination (Table 32). Neither did spore hydration cause any reduction in germination or germ tube development in the four races if the spores were kept in oil for only about one minute. However, the interaction between three or more hr of rehydration and four hr in oil completely inhibited spore germination.

A sample of spores of race 326, taken from the desiccator and kept in oil 24 hr, was tested against another sample with 24 hr rehydration plus 24 hr in oil, in their ability to infect Bond. The results indicated that the desiccated spores were able to infect Bond but that the hydrated spores caused no infection of this cultivar.

I studied the threshold of the hydration x oil interaction and found that between 1 and 2 hr of hydration at room temperature and 30 min to 1 hr in oil reduced the rate of germination, decreased germ tube length, or inhibited germination completely (Table 33).

Table 33. Average germination percentages of spores of crown rust races 216, 264A, 264B, and 326 collected and stored in a desiccator at room temperature for 48 hr, then rehydrated in a moist chamber for 0, 3, 12, or 24 hr prior to germination on water agar

Time in oil (hr)	Germination (hr)	Hours rehydration				
		0	1/2	1	2	3
.25	4	96	95	96	92	88
	24	97	98	97	94	92
.50	4	97	93	40	5	2
	24	98	97	60	8	2
1	4	97	97	30	0	0
	24	97	97	50	0	0
2	4	95	95	30	0	0
	24	97	97	40	0	0
3	4	95	95	10	0	0
	24	97	96	20	0	0

DISCUSSION

Effect of Inoculum Concentration on Mixtures of Fungus Isolates

The cultivar Bond, carrying the complementary resistance genes *Pc*-3 and *Pc*-4, was the common suscept for the races used in my experiments. Although Bond is not an isoline (but it is a parent) with respect to X-421 and C-649, it gave me the opportunity to work with a progression of resistance genes, i.e., *Pc*-3, *Pc*-4 (Bond); *Pc*-3?, *Pc*-4?, *Pc*-5 (C-649); and *Pc*-3?, *Pc*-4?, *Pc*-5, *Pc*-52 (X-421). Lines like X-421 and C-649, being near-isogenic, enable the researcher to avoid factors that might confuse interpretation of the action or effect of a given resistance gene on the rust isolate being studied.

A decrease in the number of pustules/leaf on Bond with decreasing inoculum concentration was observed in experiment 1 with all the race isolates I studied; however, race 326 or its mixtures presented the lower level of variation among inoculum concentrations and appeared to be the least affected by the range of inoculum concentrations used. Observations of X-421 and C-649 showed that infection was heavier during the second generation and that the pustule number on C-649 was twice that in the first generation; nevertheless, there was no significant difference between generations on X-421. This indicates that gene *Pc*-52, interacting with *Pc*-5 or other genes, confers upon X-421 a resistance mechanism that, up to a point, will not allow an increase in the number of lesions on X-421 even though the conditions for infection favor the high incidence of rust on other cultivars like C-649.

Both isolines X-421 and C-649 are susceptible to race 264A, which produces pustules of similar size on both of them. They responded with the same intensity of uredia of 264A in the first generation, but the number increased two fold on X-421 and four fold on C-649 during the second generation. Table 5 shows this to be highly significant, and Table 6 and Fig. 6 indicate that this was true at the high inoculum concentration. The differential effect of C-649 and X-421 on race 264A may go further than that given for gene Pc-52 alone and suggests the action of a linked pool of genes inherited from Avena sterilis that may work independently of Pc-52 to keep the number of infections down in X-421. In the field, also, X-421 is susceptible to race 264A based on visual rating of pustule type, but 264A increases and spreads in a solid stand of X-421 more slowly than one would expect of a virulent strain (J. A. Browning, personal communication). This mechanism seems similar to the slow rusting mechanism studied by Clifford (1968) that is expressed through the production of reduced numbers of pustules. Clifford (1968) considered the post-infection process to be involved in this mechanism and especially the host-parasite biochemistry and nutrition. The application of cytokinins helped the crown rust fungus maintain nutritional balance at the infection site (Clifford, 1968). The isoline X-421-race 264A system, compared with the isoline C-649-race 264A system, has fewer effective virulence genes; however, my isolate of race 264A was more aggressive on C-649 than on X-421 at higher inoculum concentrations. This result is not in agreement with Van der Plank's (1968) assumption that more ineffective genes for virulence means less aggressiveness.

The results of experiment 2 (Tables 9 and 10) indicate that race 264A is a good competitor at high inoculum concentrations and that race 216 is a good competitor at low concentrations. This result is similar to that obtained by Katsuya and Green (1967) with P. graminis tritici races 56 and 15B-1 (Can). They found that the most virulent race predominated at high inoculum concentrations. The reason for this effect is unclear but one speculation is that race 15B-1 (Can) is a stronger competitor for host nutrients when the demand for them is greatest. Race 264B can be considered a poor competitor at low inoculum concentration on isoline C-649.

The medium inoculum concentration selected for use in experiments 3 and 4 indicates (Table 9) that race 216 was at a level of less competitiveness with relation to the other isolates. The mixture between races 264A and 264B showed that the medium inoculum level was just the medium point in which 264A is increasing reciprocally with the inoculum concentration.

Effect of Temperature on Mixtures of Fungus Isolates

The high-temperature regime and the low relative humidity caused a reduction in the number of pustules that developed on Bond in treatment nine (Table 14). I observed an increase in the number of pustules/leaf in treatment 9 after the first generation and this was especially true for races 216 and 326. These facts indicate that under the high-temperature regime a selection pressure should favor the development of spores more adapted to that condition. Isolines X-421 and C-649 also presented a low number of pustules/leaf in the high-temperature treatment. These isolines were held at 24C without lamp chimneys. Thus, the reason for the low number of pustules should be attributed to the quality of spores used in the inoculation. A possible explanation of this factor is included in the discussion of the effect of oil and moisture on germination.

The effect of different temperature regimes did not present any consistent pattern; however, significant differences were observed in the magnitude but not in the general trend of the number of pustules indexed on the isolines (Figs. 8-13). The half-life calculations indicate that race 326 was the most aggressive race, under the conditions of experiment 4, and that all the other races decreased when mixed with it. Race 216 increased in the mixture with races 264A and 264B, and race 264B decreased in relation to the other races. The higher competitive ability of race 326 in relation to race 216 confirms the Browning and Frey (1969) interpretation that race group 216 is the fittest to survive in the South and race group 290 (race 326 is in race group 290) the fittest to survive in the North. However, they added that each group had enough fitness to survive in the area where the other had some advantage. The increase of race 216 relative to races 264A and 264B is in agreement with the assumption that a simple race has more fitness; however, the behavior of race 326 shows that this assumption can not be considered a generality. The performance of race 264B, under field conditions, and of my isolate of race 326.under the conditions of my studies, are examples to disprove that generality.

Comparisons between isolines and the different crown rust race mixtures were, in general, highly significant, showing the importance of the cultivar on which different races were indexed. This factor has been analyzed by several workers (Bromfield, 1967; Cournoyer, 1970; Loegering, 1951; and Torres, 1966). Race 264A predominated over race 264B on Bond in my study, but I will yield to temptation and speculate that the predominance of race 264B over race 264A should be expected if cultivars other than Bond, and especially Garland, were used to increase a mixture of these races.

The spore germination tests in experiments 1-4 (Tables 11, 12, 18, and 19) showed high germination percentages, almost all above 90%; in some cases the differences were highly significant. The high germination percentages are the result of using fresh inoculum, as pointed out by Bromfield (1967) and Loegering (1951). In the second generation of experiment 1, race 264A presented higher values than the other races. The spores with the lowest percentage germination developed in treatment 9. This decrease probably was caused by high temperature. The other germination differences should be considered as random and, as indicated by Broyles (1955), not be considered significant over a long period of time. Besides this, any difference found in this test could hardly be correlated with the infection potential. Tables 26 and 27 show that germ tube development differed significantly among the different treatments for races and among races. This indicates that, although spore viability was high, the vigor of the germ tube was different in the different treatments. The factors probably involved in this difference in vigor are discussed later in the oil-moisture effect on germination.

Leaf-wetness Duration, Infection, and Spore Production

My results correlating duration of leaf wetness and percentage of infection agreed with Marland (1938) in that I found around 5 hr of wetness necessary for infection to occur. The sparse infection observed in all the inoculated seedlings after 4.5 and 5.5 hr in the moist chamber and 40-50% relative humidity in the Plant Growth Lab indicated the effect of low relative humidity on all races. The abundant infection that races 264A and 264B showed after 14.0 hr in the moist chamber denoted the importance of free moisture for these races. The sparse infection by races 216 and 264A after 4.5 hr in the moist chamber and 70-80% relative humidity in the greenhouse pointed out the slower rate of germination and appressorium formation by these races compared to that of races 264B and 326. These results should be correlated with that for appressorium formation found by Singh (1971) where race 326 produced the most appressoria followed by races 264B, 216, and 290. Thus, races 264B and 326 have a competitive advantage during the penetration process; however, races 264A and 264B were more competitive when low relative humidity was a limiting factor during the penetration and post-penetration process.

Races 326 and 264B formed uredia larger than those of races 216 and 264A. Spore yield appeared also to be highest with race 326, but I did not include a quantitative measurement of this trait in my studies. The measurement of uredial size gives quite limited information about spore yield since, as Torres (1966) indicated, sometimes a small uredium can produce more spores than a big one. Therefore, it is risky to guess at a correlation between uredial size and spore yield. Spore production did not favor any particular race and all had the same opportunity, in time, to release their spores. The pattern of regular or irregular release should be considered an important trait, in a fluctuating environment, when the production period is similar (Browder, 1965; Torres, 1966). It also is important to keep in mind that uredial size can vary from one race to another depending on the suscept used (Torres, 1966).

The period between inoculation and the eruption of uredia was shorter in races 216, 264B, and especially race 326 than in race 264A. This was the situation at the different temperatures studied and means that increase of race 264A should be slowed down significantly relative to other races over several generations. This restriction in the fast buildup of race 264A in the population makes this race unable to use its competitive ability at high concentrations. My results are similar to those found by Browder (1965) and Katsuya and Green (1967), and they indicate that races with fewer genes for virulence seem to have a shorter period between inoculation and the eruption of uredia than those with more genes for virulence. This does not apply to the relation between races 216 and 326 but it seems true in the other cases.

The telial formation data indicate that, with the exception of race 264A, telia of races 216, 264B, and 326 developed on one cultivar or another. This should be considered an advantage for race 264A, unless cycling on the alternate host, *Rhamnus* spp., is advantageous. Race 326 formed telia in secondary pustules but the primary pustules produced uredospores through the same sporulating period as the other races. This suggests that telial formation did not affect the uredospore yield of race 326 appreciably. The formation of telia by race 216 on Landhafer and C-649 but not on Bond (Table 31) indicates that an interaction involving *Pc*-5 is conditioning this character.

Oil and Moisture Effects on Germination

The differences in infectivity of the spores of the different races during the different generations, plus the differences in germ tube development, indicated the necessity of studying some factors that might have affected the pre-germination and pre-penetration processes. The interaction between the moisture content of the spore and the time in oil showed that this interaction could repress germination at different levels or entirely according to the time spores were rehydrated and the time they were maintained in oil. The effect seems to be related to the physiological and enzymatic process that occurs during hydration, as described by Strobel (1965). Allen and Dunkel (1971) indicated that inhibitors are not present in free form in dry spores but that spores require hydration to release inhibitors in a form that is water soluble. My results showed that as soon as the biochemical and biophysical changes known to occur during the process of hydration start in the spore, the isolation of the spore from the source of water by a thick film of oil will slow down the mechanism of germination. This suggests that the normal contact of the spore with water vapor allows the spore to discharge metabolic products, and especially volatile compounds, but that spore isolation and subsequent accumulation of these products inside the spores may cause the detrimental effect observed. Differences in germ tube development (Tables 26 and 27) could have been caused by the following factors: 1) differences in the moisture content of the spores at the moment of collection and the number of spores collected; 2) differences among the spore samples after 48 hr in a desiccator; and 3) differences in moisture uptake by the spores between the time a sample was weighed and the time oil was added before inoculation. The effect invalidated any quantitative comparison among races on Bond, but it should not have affected the comparison between isolines X-421 and C-649 or the race ratios in the different mixtures, since moisture content affected those treatments equally.

These results suggest that spores should be kept in a desiccator for more than 48 hr after collection, weighed as rapidly as possible, and immersed in oil immediately to avoid moisture uptake by the spores. Oil should be useful in some spore germination studies where it is necessary to observe the accumulation of inhibitors. Also, it might be profitable to study new ways of using oil in disease control.

General Discussion

The pattern of cultivar distribution and variation, presented in Table 4 and Fig. 5, reflects a host selection pressure that is responsible, in part, for the reported decrease of races 216 and 326 and the increase of race 264B. Several cultivars susceptible to races 216 and 326 have been dropped from the population during the 5-year period, 1966-1970. Garland, resistant to races 216 and 326, did not sustain any appreciable damage from race 264A; however, race 264B seems capable of attacking and injuring this cultivar. Garland has been kept in the population, which should be an advantage for race 264B. The effect on the rust race population of the tolerant cultivars O'Brien and Nodaway is difficult to assess; however, the effect should be considered important since the tolerant cultivar Cherokee, as Torres (1966) indicated, caused a reduction in spore yield and an unevenness in the production of spores of race 216. The multiline cultivars, each consisting of several individual isolines each containing a different vertical resistance gene, have "quantitative population resistance to the rust population similar to that possessed by a pure line cultivar with tolerance or horizontal resistance" (Browning and Frey, 1969). Therefore, these multilines slow down the rate of rust increase, and each race is affected in the measure that each of the multiline cultivar components is resistant to it, or escape its attack in the case of being susceptible.

The increase in nature of race 264B at the expense of races 216 and 326, then, can be explained in part by cultivar changes, but the decrease of race 264A, which never attained a share of the rust population commensurate with its level of virulence, must be explained by other means, such as the factors I studied. The general picture that emerges from all of the factors I studied in relation to the competitive ability of my isolates of races 216, 264A, 264B, and 326 indicates that any given race will have some advantages under one set of conditions or another; however, my isolate of race 326 proved to be the fittest of my four isolates under the majority of the conditions I studied.

My isolate of race 216 was more aggressive than my isolates of races 264A and 264B. Therefore, the decrease of race groups 216 and 290 (including race 326) in the field population probably would not have happened without host selection pressure. The decrease of race 264A was to be expected independently of host cultivars, however. Results with my isolate of race 264B are not in agreement with the field data about that race. This could be because my isolate was not adequately representative of the 264B population in nature or, if my isolate was representative, the results could be an artifact of my techniques (such as harvesting spores only once, 14 days after inoculation) that did not allow my 264B isolate to show its true competitive abilities.

Race 326 is considered to have at least five more genes for virulence than race 216 (Table 1) and theoretically it should be less fit than 216 to survive on Bond. Virulence generally is inherited as a recessive trait and races with fewer genes for virulence should have more heterozygosity (Flor, 1942). Dinoor (1969) selfed isolates of the most common crown rust races in Israel, including some that are highly virulent, and found that they appeared to be heterozygous. He considered this to be the cause of the amplification of the pathogenic variability and maybe of the aggressiveness of crown rust in Israel. Therefore, it would be helpful to know the inheritance of those genes controlling virulence to establish if, in fact, they are all inherited as recessive traits. If heterozygosity can not be correlated with aggressiveness, it should be considered that there is not any positive correlation between virulence and aggressiveness and that aggressiveness is inherited independently of virulence and at random.

The samples of the pathogen I studied were infinitesimally small in comparison with the population in nature, and the same could be said relative to the few environmental factors it is possible for one to study in the laboratory and the many ecological factors that influence the competitive ability among races through consecutive generations in nature. Plant growth chamber studies enable one to measure quantitative differences among competing races and produce valid differences enabling one to evaluate competitive ability under given, restricted sets of environmental conditions, but these can only be minimally suggestive of what actually happens in nature. Still, man must enter the vastness of nature where he can, learn what he can, and project this knowledge, realizing its limitations, to the larger picture. This I have attempted to do.

SUMMARY AND CONCLUSIONS

In an attempt to help explain racial dynamics observed in nature, I conducted four studies to evaluate the effect of inoculum concentration and temperature on the components of mixtures of isolates of four *Puccinia coronata* var. *avenae* races competing on a susceptible oat cultivar. I studied also the effect of leaf wetness duration, onset and duration of urediospore production, telial formation, and the interaction of oil and spore hydration on spore germination.

I selected one monouredial culture of each of four races, 216, 264A, 264B, and 326, of *P. coronata avenae*. Three day/night temperature regimes (21/12, 26/17, and 32/19 C) and three inoculum concentrations (low, medium, and high) were imposed on the cultivar Bond (that is fully susceptible to the four races) inoculated with my isolates for 14-day generations. The infection on Bond by each race in each mixture each generation was indexed on the isolines X-421 and C-649. The inoculated seedlings were maintained in plant growth chambers. The two main characters measured were number of uredia/leaf and the ratio of resistant to susceptible infection types. I also recorded percentage of spore germination, the period between inoculation and the eruption of uredia, size of sporulating area, duration of spore production, and degree of telial formation. The half-life of each decreasing race, in the concept of Van der Plank (1968), was determined for isolates in the different race mixtures.

Differences existed among the four races according to the inoculum concentration used. Cultivar X-421 presented a resistance mechanism that, up to a point, did not allow an increase in the number of lesions even though the conditions for infection duplicated those favoring a high incidence of rust on C-649. Compared with the isoline C-649-race 264A system, the isoline X-421-race 264A system has fewer ineffective genes for virulence; however, race 264A was more aggressive on C-649 than on X-421 at the high inoculum concentration. My isolate of race 264A was a good competitor at high inoculum concentrations and race 216 was good at low concentrations.

Half-life calculations indicate that race 326 was the most aggressive race under the conditions of experiment 4, and that all the other races decreased when in mixtures with it. Race 216 increased in the mixture with isolates of races 264A and 264B, and race 264B decreased in relation to the other races. Differences due to temperature and moisture indicate that the low temperature regime induced an increase in the number of pustules compared to the medium or high temperature regimes. Race 264A was favored by the 26/17C day/night temperature regime. The consistent trend in the half-life pattern did not change with differences in temperature or with the isoline used; however, isolines differed significantly in the infection ratios and that changed the magnitude but not the trend.

Spore germination was high; uredospores from almost all treatments germinated above 80%. Low germination was correlated with high temperature. Differences in inoculum concentration did not change spore germination significantly. Some differences were random among my four isolates and they should not be considered biologically important over a long period of time.

My isolates of races 264B and 326 had competitive advantage during the penetration process because they appeared to germinate faster than the other isolates; however, races 264A and 264B were more competitive when low relative humidity was a limiting factor following a period of free moisture. The period between inoculation and the eruption of uredia favored races 216, 264B, and especially race 326, over race 264A. Thus, inoculum increase of race 264A would be slowed down significantly over several generations if my isolate typified that race. Uredia of races 326 and 264B were larger than those of my isolates of races 216 and 264A.

From visual observations, my isolate of race 326 sporulated more profusely than the others. Only race 264A failed to produce telia, but this advantage did not affect the other races since the telia developed as secondary sporulation and not in the primary uredia.

I found an interaction between the rapid uptake of moisture by crown rust spores and the time spores were in oil before inoculation or spore germination tests. Oil reduced the vigor and viability of rehydrated uredospores and in some cases germination was repressed completely. This suggests that as soon as the biochemical and biophysical changes known to occur during spore hydration start, the isolation of the spore from a source of moisture causes a reduction in germination. I will speculate that free atmospheric moisture acts as a sink for some of the spore's volatile metabolic by-products and that isolation of the spore in the oil causes the accumulation of these products inside the spore which has detrimental effects.

Examination of data relevant to changes in the oat cultivar population indicated a host selection pressure that has been responsible, in part, for the reported decrease of races 216 and 326 and the increase of race 264B. Results with my four isolates suggest that race 326 is the fittest race to survive under the majority of the conditions I studied. My isolate of race 216 was more aggressive than my isolates of races 264A and 264B. Therefore, the decrease of race groups 216 and 290 (including race 326) in the population probably would not have happened without host selection pressure. The decrease of highly virulent but weakly aggressive race 264A was to be expected independently of host cultivars, however. The results with my isolate of race 264B are not in agreement with the field data about 264B. It should be considered that my isolate may not have represented the 264B population in nature; however, if it did, then my results are an artifact of the techniques used that did not cover the conditions that would allow my isolate to use its true competitive abilities.

The highly significant difference between isolines X-421 and C-649 emphasized the importance of any particular host in race competition. They indicate also that quantitative studies, with different inoculum concentrations, should be useful in selecting slow rusting lines or cultivars tolerant to crown rust.

These facts suggest that, under the conditions of my studies, the race with fewer genes for virulence will not always be the more aggressive. A study of the inheritance of genes controlling virulence should aid understanding since aggressiveness has been correlated in several pathogens with the heterozygosity of the different races. If this is not the case it should be considered that there is not any positive correlation between virulence and aggressiveness and that aggressiveness is inherited independently of virulence and at random.

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ACKNOWLEDGEMENTS

I am indebted to the Rockefeller Foundation and to the Colombian Agricultural Institute (ICA) who made it possible to me to undertake graduate education abroad, and to Iowa State University for supporting my research at that institution.

I am grateful to my committeemen: Drs. J. A. Browning (chairman), M. D. Simons, R. H. Shaw, R. E. Atkins, and C. E. LaMotte for their advice and help in my research endeavors. I am also very grateful to Gayle Browning, Roland Barker, and John Thomas, for their valuable help in the accurate recording of my data, and to Edwin L. James, Robert F. Metzner and Clifton G. Prange for their cooperation and opportune help in "running the interference" in dealing with the refrigeration system in the Plant Growth Labs.

The author wishes to express his sincere appreciation for the counsel, encouragement, constructive criticism, and friendship offered by Dr. J. A. Browning during the course of this study and in the preparation of the manuscript.

Appreciation is due to my wife, Edith, and my daughters, Gloria Maria and Angela Beatriz, for keeping me alive with their love, patience and understanding during the years of my study.

Special debts of gratitude are due to my late and forever beloved mother, Alicia. She was the wonderful human being who guided the first steps in my life and who subsequently gave me lessons of sacrifice, hard work, and perseverance -- the only course a mother of the middle-class can pursue in getting her son educated.