ENVIRONMENTALLY INDUCED VARIABILITY IN THE HOST REACTION OF BARLEY TO NET BLOTCH

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Summary

The host reaction of two Ethiopian and four Manchurian resistant varieties, and one susceptible variety of barley to isolate 177 of *Drechslera teres* was studied under a range of environmental conditions. The effects were specific to the variety(ies) used, with the exception of the factor of post-inoculation temperature. High post-inoculation temperatures brought about the breakdown of resistance in all the varieties.

The expression of the resistance was more sensitive to environmental influence in varieties of Manchurian origin than in Ethiopian varieties. All Manchurian varieties expressed a higher degree of resistance with age. The resistance of these varieties was enhanced by providing either pre-inoculation high temperatures, or high light intensities during the incubation period. Variations in the concentration of conidial suspension and age of the subculture also affected the reaction of Manchurian varieties.

The studies of the host reaction under a range of environmental conditions have been considered to provide better understanding in the genotype–phenotype correspondence, thereby enhancing the genetic interpretation.

I. INTRODUCTION

Net blotch, caused by *Drechslera teres* (Sacc.) Shoem., is an important disease of barley (Khan, Boyd, and Shipton 1968). Considerable work has been done in the search for resistant barley varieties (Schaller and Wiebe 1952; Buchannon and McDonald 1965; Dessouki, Mansour, and Khalifa 1965). It has been frequently observed while reviewing such literature that certain varieties, which proved highly resistant in some reports, have given intermediate, susceptible, or inconsistent reactions in others. Whilst the possibility exists that such differences may have been contributed by the variation in the pathogenic properties of the local isolates, the role of environmental influence on variation in host reaction has also been indicated in the literature (Singh 1956).

With regard to temperature, Singh (1963*a*) has shown that, although optimum temperatures for infection and lesion development were 25 and 29°C respectively, infection can occur over a wider temperature range (8–33°C). No detailed studies on the effect of light are available, although exposure to ultraviolet radiation has been shown to cause an increase in the severity of the symptoms (Chakrabarti 1968). It has also been reported that pre- and post-inoculation wetting enhance infection. In the case of post-inoculation wetting, the minimum requirement varied from 10 to 30 hr depending upon the isolate used (Singh 1963*b*).

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There are reports that some agronomic treatments may affect host reaction to net blotch; for example, nitrogen application has been shown to increase the severity of the disease (Bönning and Wallner 1934; Singh 1963*d*). On the other hand, Piening (1967) indicated that this nitrogen effect is not always observed. The role of other major and minor nutritional elements has been studied by Singh (1963*d*). He found that deficiencies of calcium, phosphorus, iron, magnesium, potash, and sulphur resulted in reduced infection.

The experiments described in this paper were carried out to study the effect of certain climatic and biotic factors on the host reaction of barley to net blotch, as a preliminary to studies on the inheritance of host resistance.

II. Methods and Materials

One susceptible, and six resistant (including inconsistently resistant) varieties used in these investigations are listed in Table 1. The seeds were obtained from Dr. K. W. Buchannon, Canada Department of Agriculture, Winnipeg, Canada, and International Barley Disease Nursery. Due to insufficient seeds being available, all varieties have not been included in each experiment.

Table 1

BARLEY VARIETIES USED IN VARIOUS EXPERIMENTS

Variety	C.I. No.	Origin	Resistance to Net Blotch
Dampier W.45	13521	Hybrid	Susceptible
Harbin	4929	Manchuria	Resistant
Manchuria	2330	China–Manchuria	Resistant
Tifang	4407–1	Manchuria	Resistant
Ming	4797	Manchuria	Resistant
—	5791	Ethiopia	Resistant
—	9819	Ethiopia	Resistant

(*a*) *Temperature Control*

Throughout the course of the investigation, plants were grown in air-conditioned glasshouse rooms, where programmed temperatures ranged from 18°C minimum at night to 24°C maximum during the day. This temperature regime is considered as the "standard" temperature condition. In experiments involving the effect of temperature treatments, two additional levels ("low" and "high") were used, in which the daily maximum was 18 and 36°C respectively.

(*b*) *Light Control*

All experiments conducted in the glasshouse rooms received approximately 12 hr of natural daylight. After inoculation, plants were maintained under high humidity for 48 hr. This period has been referred to as the "incubation" period. During this time reduced light conditions ($5 \cdot 38$–$10 \cdot 76 \times 10^4$ lux) were imposed by providing shade covers.

In experiments involving the effect of light intensity during the incubation period, three additional light treatments were employed. These were provided in Arcus growth cabinets in which standard temperature conditions (± 1°C) were maintained. The light treatments were:

(1) $38 \cdot 75 \times 10^4$ lux for 12 hr per day;
(2) $5 \cdot 38 \times 10^4$ lux for 12 hr per day;
(3) Complete darkness continuously for 48 hr.

(c) *Technique of Inoculation*

Monoconidial isolate 177 was used in all the experiments. Subcultures were grown on V-8 Juice agar at 15°C under continuous light. Conidial suspensions were prepared from 15-day-old subcultures and standardized at 10,000 conidia per millilitre.

Plants were grown in 6-in. clay pots and were regularly watered with a balanced nutrient solution. Twenty plants were tested for each treatment.

Seedlings, 15 days old, were sprayed with a standardized conidial suspension. Thereafter they were covered in wet plastic bags and maintained at 95–100% R.H. under reduced light conditions ($5 \cdot 38$–$10 \cdot 76 \times 10^4$ lux) for 48 hr. After inoculation, plants were transferred to glass-house benches and maintained at standard temperature conditions. The inoculation procedure outlined above is referred to as the "standard inoculation technique".

(d) *Scoring*

The scoring of disease reactions was carried out 14 days after inoculation, using the five-class scale described by Khan and Boyd (1969, p. 1231). Mesothetic reactions were also indicated in the manner described by these authors.

III. Results

(a) *Effect of Pre- and Post-inoculation Temperatures*

No major changes were observed at low and standard pre- or post-inoculation temperatures (Table 2). Variety Dampier exhibited consistently susceptible reactions over the entire range of temperatures.

TABLE 2

REACTION OF BARLEY VARIETIES TO INFECTION WITH ISOLATE 177 OF *D. TERES* FOLLOWING EXPOSURE TO DIFFERENT PRE- AND POST-INOCULATION TEMPERATURES

Reactions are scored as described by Khan and Boyd (1969)

Variety	Pre-inoculation Temperature:			Post-inoculation Temperature:		
	Low	Standard	High	Low	Standard	High
Dampier	4	4	4	4	4	4
Manchuria	X(3,1,2)	X(3,1)	1	X(3,1)	X(3,1)	X(3,1,odd 4)
Harbin	X(2,3)	X(2,1,3)	1	—	—	—
Tifang	—	—	—	X(1,2)	1	X(1,odd 4)
C.I. 5791	1	1	1	1	1	X(1,odd 4)
C.I. 9819	1	1	1	1	1	X(1,odd 4)

Varieties C.I. 5791 and C.I. 9819 exhibited 1-type reaction at all the pre-inoculation temperatures. In contrast, Manchuria and Harbin gave variable reactions. Following low and standard pre-inoculation temperatures, predominantly moderately resistant to moderately susceptible types were observed. However, pre-inoculation high temperature induced a high degree of resistance in both of these varieties.

At high post-inoculation temperatures a general breakdown of resistance was observed. Odd lesions, showing 4-type reactions, were observed in almost all the resistant varieties. The susceptible variety Dampier, which did not exhibit any change in reaction type, showed comparatively rapid development of symptoms at high temperatures.

(*b*) *Effect of Light Treatments during Incubation*

Each of the five barley varieties were maintained at three light intensities ($5 \cdot 38 \times 10^4$, $10 \cdot 76 \times 10^4$, and $38 \cdot 75 \times 10^4$ lux) during the incubation period. Each treatment was replicated twice.

The results given in Table 3 show that the reactions of Dampier, C.I. 5791, and C.I. 9819 were not affected by the light treatments. Manchuria and Ming, however, exhibited extreme variability of reaction type. A breakdown of resistance was observed as the light intensities were reduced. Manchuria was the most sensitive variety.

TABLE 3

REACTIONS OF FIVE BARLEY VARIETIES INFECTED WITH ISOLATE 177 OF *D. TERES* AND SUBJECTED TO DIFFERENT LIGHT TREATMENTS DURING THE 48 HR OF THE INCUBATION PERIOD

Reactions scored as in Table 2

Variety	Light Intensity (lux)		
	$38 \cdot 74 \times 10^4$	$5 \cdot 38 \times 10^4$	0
Dampier	4	4	4
Manchuria	X(1,2)	X(3,2,1,4)	X(4,3)
Ming	1	X(1,2)	X(4,3)
C.I. 5791	1	1	1
C.I. 9819	1	1	1

TABLE 4

AVERAGE NUMBER OF LESIONS PER SQUARE CENTIMETRE OF LEAF AREA IN THREE BARLEY VARIETIES UNDER THREE LIGHT INTENSITIES DURING THE INCUBATION PERIOD

Variety	No. of Lesions at Light Intensity (lux) of:			Mean*
	$38 \cdot 75 \times 10^4$	$5 \cdot 38 \times 10^4$	0	
Dampier	3·541	2·325	0·969	2·270
Ming	3·317	1·466	0·708	1·830
Manchuria	2·564	1·191	0·481	1·410
Mean*	3·140	1·660	0·719	1·839

* Least significant difference = 0·50.

The number of lesions developed on the first leaf of each of the plants of varieties Dampier, Manchuria, and Ming was counted, and an analysis of variance of the results was carried out. Significant differences between varieties and between light treatments were obtained. In all varieties, the number of lesions increased progressively with a decrease in the light intensity (Table 4). It was found that the

resistant varieties Manchuria and Ming had smaller numbers of lesions than the susceptible variety Dampier. This supports the report by Keeling and Banttari (1965) who found that susceptible varieties develop more lesions than those which are resistant.

(*c*) *Effect of Age of Host*

Reactions of seven varieties inoculated at different ages are presented in Table 5.

TABLE 5

REACTION OF BARLEY VARIETIES INOCULATED WITH ISOLATE 177 OF *D. TERES* AT DIFFERENT AGES

Reactions scored as in Table 2

Age of Host (days from sowing)	No. of Leaves at Inoculation	Reaction of Variety: Dampier	Harbin	Manchuria	Ming	Tifang	C.I. 5791	C.I. 9819
10	1	4	3	X(3,1)	X(1,2)	X(1,2)	1	1
15*	2	4	X(3,2)	X(3,2,1)	X(1,2)	X(1,2)	1	1
20	2–3	4	X(3,2,1)	X(3,2,1)	X(1,2)	1	1	1
25	3–4	4	X(3,2)	X(3,2,1)	1	1	1	1
50–60†	—	4	X(2,1)	X(2,3,1)	1	1	1	1

* Age of host used in the standard inoculation technique. † Heading stage.

An increase in the expression of resistance was observed with increasing age in the varieties Harbin, Manchuria, Ming, and Tifang. This acquisition of greater resistance with increasing age was more pronounced in Tifang and Ming than in Harbin and Manchuria. Visual observations indicated a smaller number of lesions following inoculation at the heading stage than were apparent from infection at the seedling stage.

(*d*) *Effect of Age of Pathogen*

Identical quantities of the standardized conidial suspension prepared from 10-, 15-, 20-, and 25-day-old subcultures of *D. teres* grown on V-8 Juice agar in 50-ml conical flasks were used to inoculate four barley varieties. The results are presented in Table 6.

The susceptible variety Dampier and resistant varieties C.I. 5791 and C.I. 9819 gave consistent reactions to the inoculum treatments. However, the variety Manchuria gave predominantly resistant reactions (1 and 2 types) to inoculum obtained from 10-day-old cultures, but more susceptible-type reactions to inoculum prepared from older cultures. It would seem, in the case of this variety, that conidia obtained from 10-day-old cultures were not sufficiently mature for successful infection, while older cultures produced more infective conidia.

(e) *Effect of Concentration of Conidial Suspension*

The reaction of four barley varieties to inoculations with conidial suspensions containing 5, 10, 20, and 40 thousand conidia per millilitre are shown in Table 7.

TABLE 6

REACTIONS OF BARLEY VARIETIES TO INFECTION WITH INOCULUM OBTAINED FROM SUBCULTURES OF DIFFERENT AGES

Reactions scored as in Table 2

Variety	Age of Subculture (days)			
	10	15*	20	25
Dampier	4	4	4	4
Manchuria	X(1,2)	X(3,2,1)	X(3,2)	X(3,2)
C.I. 5791	1	1	1	1
C.I. 9819	1	1	1	1

* Age of subculture used in the standard inoculation technique.

TABLE 7

REACTIONS OF BARLEY VARIETIES FOLLOWING INOCULATION WITH DIFFERENT CONCENTRATIONS OF CONIDIAL SUSPENSIONS OF ISOLATE 177 OF *D. TERES*

Reactions scored as in Table 2

Variety	$10^{-3} \times$ Concentration of Conidial Suspension (conidia/ml)			
	5	10*	20	40
Dampier	4	4	4	4
Manchuria	X(1,2)	X(3,1)	3	X(3,4)
Tifang	1	1	X(1,2)	1
C.I. 5791	1	1	1	1

* Concentration of conidial suspension used in the standard inoculation technique.

The reactions of varieties Dampier, Tifang, and C.I. 5791 were not affected by the different spore concentrations. A breakdown in the resistance of variety Manchuria was observed with increasing concentrations of conidial suspension.

IV. Discussion

The effect of almost all the factors studied was specific to the variety or varieties used, with the exception that post-inoculation high temperatures caused a general tendency to the breakdown of resistance. The susceptible variety Dampier gave consistent reactions over the entire range of environmental and biotic conditions imposed. Similarly, resistant varieties from Ethiopia, C.I. 5791 and C.I. 9819, consistently exhibited resistant reactions. Varieties from Manchuria, however, provided variable reactions under different conditions tested. This was particularly so with the variety Manchuria. The results indicate the differences in the genetic basis for host reaction among resistant varieties. These differences could be due to differences in their genetic background (Boyd 1966), or to allelic differences amongst specific genes for resistance (Martens, McKenzie, and Green 1967).

The two most important environmental factors to affect the host reaction of Manchuria were found to be pre-inoculation temperature and light intensity during incubation period. Manchuria, which exhibits variable reactions under standard conditions, may be induced to exhibit uniform resistant reactions by provision of either (1) high pre-inoculation temperatures or (2) high light intensities during incubation. It would appear that the pre-inoculation temperatures may affect some of the preliminary steps in the resistance mechanism. However, light intensity during the incubation period would seem to involve the host resistance which develops after the physiological contact between the host and parasite. The possibility that light conditions influence the parasite itself is doubted, since we observed that reduced light discourages the growth of *D. teres*. Piening (1964) found that a reduction in an antifungal compound occurred in the leaf homogenates of a resistant barley maintained under dark conditions, and this supports our findings.

The increase in resistance with age, such as that observed in the present study, corresponds with the report by Piening (1964), who showed that an antifungal substance in barley leaves increased in concentration as plants became older. Singh's work (1963*c*) showed that the incidence of net blotch is governed by the interaction between the age of host and the isolate of the pathogen. His work, however, was limited to 33-day-old plants.

The breakdown in the resistance of the variety Manchuria on inoculation with more concentrated conidial suspension may have been caused by a high "inoculum potential" achieved by heavy "spore-load" (Garrett 1960). The same argument may also possibly hold for the effect of age of the pathogen, where Manchuria exhibited a high degree of resistance on inoculation with a conidial suspension obtained from 10-day-old subcultures. Ten-day-old subcultures may have provided a greater proportion of immature and non-infective conidia than the older subcultures, resulting in a lower "effective" concentration of the suspension. However, our results on the age of the pathogen do not agree with those of Singh (1963*c*), who found that 10-day-old subcultures provided the inoculum of highest infectivity. This discrepancy might have been caused by differences in the techniques of culturing this fungus.

The results reported here clearly show that the consistency of the expression of host resistance to net blotch is a varietal characteristic, and that inconsistently

resistant barley varieties (such as Manchuria) are sensitive to a wide range of modifying influences.

A study of the expression of host resistance under a range of environmental conditions should provide a better understanding of the correspondence between genotype and phenotype, thereby enhancing the interpretation of genetic studies.

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VI. References

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