COTYLEDON LIMITED INFECTION (CLI) AND LEAF DISK IMMERSION (LDI) INOCULATION OF SUNFLOWER BY DOWNY MILDEW (Plasmopara halstedii)

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SUMMARY

Sporulation of the downy mildew pathogen (Plasmopara halstedii) on the cotyledons of inoculated sunflower seedlings was long accepted as evidence of susceptibility, until it was shown that sparse sporulation might occur on resistant seedlings without progressing above the cotyledonary node. Profuse sporulation limited to the cotyledons (CLI) has been observed in some host-genotype pathogen-race combinations, and recently has been shown to occur particularly when low concentrations of inoculum were used. Inoculations were made by the WSI method on pre-germinated seedlings of sunflower lines carrying no known resistance genes, or Pl 1, Pl 2, Pl 2,5, and a few seedlings of two unknown genotypes from crosses with HA 61 in some work on rust resistance, using North American races 1, 2, 3, and 4 (International races 0, 1, 2, and 2,5 respectively) at 50,000 zoosporangia per mL. CLI occurred on approximately 20% of the seedlings in a few compatible combinations where most showed systemic infection; it occurred with all four races on the two lines derived from HA 61. Uninoculated plants in the same series of experiments were inoculated by the LDI method with each of the four races using disks from leaves and from cotyledons. In most combinations sporulation was as profuse on leaf disks as on cotyledons, apparently substantiating the hypothesis that CLI was attributable to a mechanical, rather than a physiological barrier at the cotyledon node. The evidence was not consistent, however, because sporulation also occurred on leaf disks in combinations which were incompatible in WSI inoculations, although the

calculated infection index was higher in compatible than in incompatible combinations, except for the derivatives of HA 61.

INTRODUCTION

Downy mildew Plasmopara halstedii (Farl.) Berl. & De Toni infection on sunflowers is recognized in the field by systemic symptoms on affected plants. When pre-germinated sunflower seeds of susceptible lines are inoculated under suitable conditions, systemic infection usually results. In most incompatible host-genotype - mildew-race combinations, no sporulation develops on seedlings inoculated by the WSI method (Cohen and Sackston 1973). In some incompatible combinations, sparse sporulation may occur on the cotyledons but not on the true leaves (Montes and Sackston 1974, Viranyi and Dobrovolszky 1980). The apparent barrier to mildew development beyond the cotyledonary node appears to be mechanical (Allard 1978, Viranyi and Mohamed 1985).
In certain host-pathogen combinations sporulation may be profuse on the cotyledons, with no apparent symptoms developing on the true leaves. This interaction was termed cotyledon-limited systemic infection (CLS) by Ljubich (1988), and cotyledon limited infection (CLI) by Gulya (1991). It is characteristic of the reaction of differential lines carrying the Pl 2+Pl 5 genes for resistance, when inoculated with downy mildew race 1,2,5(CL) (North American race 4) (Ljubich et al 1988). Gulya (1991) inoculated seedlings of a differential line carrying resistance gene Pl 2 with varying concentrations of race 1,2 (North American race 3), a compatible combination. He found that the percentage of seedlings showing any symptoms of downy mildew decreased markedly with decreasing spore concentration from 20,000 to 200 zoosporangia per ml. The proportion of infected seedlings showing only CLI increased significantly at spore concentrations below 6 zoosporangia/mL.

This paper reports an attempt to determine if races of the downy mildew pathogen, unable to penetrate beyond the cotyledons of sunflower seedlings in incompatible combinations inoculated by

WSI, can infect the true leaves of those genotypes when the leaf disk immersion (LDI) method of inoculation (Sackston and Vimard 1988) is used. Such inoculation avoids any mechanical barrier which might exist at the cotyledonary node.

Materials and Methods

The host plants used and the genes for downy mildew resistance they possess, were as follows:

No known resistance genes:
Peredovik, open pollinated cultivar.
IS 003, hybrid line developed by Interstate Seed Co., Fargo, ND.

Gene Pl 1, conferring resistance to NA race 1:
CM 5RR and CM 90RR, both inbred lines developed at Agriculture Canada Research Station, Morden, Man.

Gene Pl 2, conferring resistance to NA races 1 and 2:
RHA 274, inbred line developed by USDA, Fargo, ND.
IS 7000, inbred line developed by Interstate Seed Co., Fargo, ND.

Genes Pl 2,Pl 5, conferring resistance to NA races 1,2, and 3:
IS 2000 and IS 3003, both inbred lines developed by Interstate Seed Co.

Genotype unknown; CLI with NA races 1 and 2, CLI or no symptoms with NA races 3 and 4:
No. 35, and No. 36, sister lines produced in the greenhouse at Macdonald College from crosses with HA 61. Only a few seeds of each line were available.

Seed of Peredovik, CM 5RR, and CM 90RR, was obtained from Dr. W. Dedio, Agriculture Canada Research Station, Morden, Manitoba. Seed of RHA 274, and of the IS lines was obtained from Dr. T.J. Gulya, US Department of Agriculture, Fargo, ND.

Downy mildew races:

Four North American races were used. Their international designations are given in parentheses: 1 (0); 2 (1); 3 (1,2); and 4 (1,2,5(CL) (Sackston et al 1990).

Inoculations:

WSI inoculations (Cohen and Sackston 1973) were made using 50,000 freshly harvested zoosporangia per mL of suspension. Ten inoculated seedlings per pot were planted in a commercial peat-vermiculite soil substitute (Promix) and placed in controlled environment cabinets maintained at 20°, with 16 hour daylength, for 13 days. They were then covered with plastic bags to achieve 100% relative humidity overnight, and mildew infection on the cotyledons and on the leaves was rated on a scale from 0 to 5. There were three replicates per treatment.

LDI inoculations (Sackston and Vimard 1988) were made on the same days as the WSI inoculations. Disks 10 mm in diameter were cut from cotyledons and from leaves of uninoculated 2-week-old plants of the same lines used in WSI inoculations, and immersed in spore suspensions of the same concentration, and incubated under the same conditions, as the WSI material. After incubation disks were placed on 2% water agar, 10 disks per 100 mm petri dish, and incubated for 7 to 10 days at 15°. Sporulation was then assessed on a 0 to 5 scale, using a dissecting microscope. There were two replicates per treatment.

Infection index and sporulation index:

To facilitate direct comparison between severity of mildew infection on seedlings, and sporulation on cotyledon and leaf disks, indices were calculated based on the numbers of individual seedlings or disks with each level of disease or of sporulation, and adjusting the values so that the maximum would be 100.

Due to unforeseen circumstances it was not possible to repeat the experiment several times as planned; this is therefore a preliminary report.

RESULTS AND DISCUSSION

The intensity of systemic infection and of CLI respectively in each of the host-genotype pathogen-race combinations inoculated by WSI is shown in Table 1. The intensity of sporulation on disks taken from leaves and cotyledons respectively in each of the host-genotype pathogen-race combinations is shown in Table 2. The data were too limited for any statistical significance to be inferred.

CLI may result when the spore concentration used for inoculation is low, as shown for WSI by Gulya (1991). Incidence and intensity of sporulation in inoculations by LDI is also affected by concentration (Sackston and Vimard 1988). Low spore concentration was not a factor in these experiments.

A low incidence of CLI could be expected in some incompatible combinations, as in CM 5RR with race 1. CLI was too high to be readily explicable in RHA 274 with race 2. CLI was not expected in the combinations CM 5RR with race 4, or in IS 7000 with races 3 and 4; it may perhaps be attributable to some unnoticed deficiency in environmental conditions during incubation. The consistent CLI infection with all four races on the two lines from crosses with HA 61 was very interesting. It is unfortunate that there is no more seed of these lines for further testing.

The very high systemic infection by race 4 on ISI 2000 and ISI 3003 was quite unexpected. CLI by race 4 on IS 2000 was used by Ljubich et al (1988) to distinguish it from race 5, which caused systemic infection. Race 4 used in these studies was provided originally by Gulya several years ago. There are only the four races in the downy mildew collection in my laboratory, so either there was an undetected admixture in the isolate as received, or some change has occurred in my isolate of race 4. This possibility will be investigated in future.

Some difficulties occurred with the LDI inoculations, unexpectedly low sporulation in some combinations, and severe bacterial contamination of the disks in others. Attempts to overcome such problems have been reported elsewhere (Sackston and Anas 1991, Helia, in press). Bacterial contamination may have been responsible, at least in part, for the low sporulation ratings of cotyledon disks of IS 2000 inoculated with race 3, and CM 5RR inoculated with race 4.

Some of the results are interesting, despite the limitations of the experiment. Ratings of systemic infection in WSI and sporulation on leaf disks in LDI were high for both lines carrying gene Pl 0,inoculated with races 1 and 3. There was no systemic infection in WSI or sporulation on disks in LDI inoculations of the lines carrying gene Pl 2,inoculated with race 1; systemic infection in WSI and sporulation on leaf disks inoculated with race 3 was high for both lines (Table 1). These combinations of host genes and pathogen races were the same as those used in the original work which indicated the potential value of LDI as a non-destructive means of determining the reaction of individual sunflower plants to various races of downy mildew (Sackston and Vimard 1988); the results corroborate that work . They also seem to indicate that failure of the pathogen to develop above the cotyledons in incompatible combinations is attributable to resistance of the epicotyl and true leaves, and not just to a barrier at the cotyledonary node.

The correlation between results of WSI and LDI inoculations did not seem to hold in some of the other cases. The two lines carrying gene Pl 2 showed no systemic infection when inoculated by WSI with race 2, but a low to medium sporulation index on both leaf and cotyledon disks when inoculated by LDI. The same relationship held for the lines carrying genes Pl 2,5; no systemic infection when inoculated with race 3 by WSI, but appreciable sporulation on both cotyledon and leaf disks when inoculated by LDI (Table 2). Apparently the correlation between

Table 1. Intensity of infection of sunflowers inoculated by WSI with four North American races of downy mildew

Host Line	Resist Gene	Race 1		Race 2		Race 3		Race 4	
		Sys	CLI	Sys	CLI	Sys	CLI	Sys	CLI
Pered	0	97	3	100	0	86	4	79	7
IS003	0	91	9	100	0	67	0	90	10
CM5RR	1	0	7	81	19	87	7	47	23
CM90RR	1	27	32	100	0	80	10	50	0
RHA274	2	0	0	0	63	100	0	100	0
IS7000	2	0	0	0	0	63	23	53	17
IS2000	2,5	0	0	0	0	0	0	89	5
IS3003	2,5	3	0	0	0	0	3	79	18
No.35	?	0	100	0	100	0	100	0	75
No.36	?	0	17	0	33	17	83	0	8

Sys = systemic infection. CLI = cotyledon limited infection.

Values obtained by multiplying intensity of infection (0 to 5) per plant by number of plants in each category and converting the sum to per cent of maximum possible.

Table 2. Intensity of sporulation on sunflower leaf and cotyledon disks inoculated by LDI with four North American races of downy mildew

Host Line	Resist Gene	Race 1		Race 2		Race 3		Race 4	
		Leaf	Cot	Leaf	Cot	Leaf	Cot	Leaf	Cot
Pered	0	55	64	59	66	84	86	72	70
IS003	0	77	20	58	70	90	76	55	66
CM5RR	1	21	46	41	66	84	44	85	28
CM90RR	1	34	54	63	80	84	86	87	86
RHA274	2	0	0	44	42	81	88	93	100
IS7000	2	0	0	15	70	82	62	83	90
IS2000	2,5	0	2	40	48	26	0	93	94
IS3003	2,5	2	4	11	44	34	22	87	96
No.35	?	76	80	74	88	63	44	80	-
No.36	?	13	76	62	90	80	86	-	-

Values obtained by multiplying intensity of sporulation (0 to 5) per disk by number of disks in each category and converting the sum to per cent of maximum possible.

(-) indicates no disks available, or rating impossible because of bacterial contamination.

results of WSI and LDI inoculations is dependent upon the specific combination of host resistance genes and pathogen races. Line No. 35 showed no systemic infection but consistently high levels of CLI infection when inoculated with any of the four races by WSI. The sporulation index was high or moderately high on all leaf disks inoculated with any of the four races by LDI, and on cotyledon disks inoculated with races 1, 2, or 3; no readings could be made on cotyledons inoculated with race 4. The results were less consistent with line No. 36, but the trend was the same. At least in these combinations, failure of the respective races of the pathogen to penetrate beyond the cotyledons to the true leaves in seedlings inoculated by WSI seemed to be attributable to a barrier at the cotyledonary node, rather than to resistance in the epicotyl or leaf tissues.

Extensive additional work will be required to determine whether the relationlships reported above were accidental or real, and whether they apply also in other host-race combinations, or are restricted to the specific resistance genes and races studied. The results of such additional studies may help to select materials suitable for the study of the nature of sunflower resistance to downy mildew.

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