COMPONENTS OF RATE-REDUCING RESISTANCE TO CERCOSPORA LEAF SPOT IN SUGAR BEET: INCUBATION LENGTH, INFECTION EFFICIENCY, LESION SIZE

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SUMMARY

Four sugar beet cultivars exhibiting increasing levels of rate-reducing resistance towards *Cercospora beticola* ('Cyrano' or 'Univers', 'Bushel', 'Monodoro', and 'Break') were used in monocyclic infection experiments to study the following resistance components: incubation length (IP, the degree-day cumulation, base 5°C, during the time between inoculation and appearance of spots), infection efficiency of conidia (INF, the number of necrotic spots per cm^2 of leaf at the end of a single infection cycle), and lesion size (LS, the area of single spots). All the resistance components were influenced by cultivar significantly; whereas no differences in lesion type were found. Resistance delayed the appearance of spots by a maximum of 12 days compared to the susceptible cultivar. IP_{50} was delayed by a degree-day cumulation of 28°C, 60°C and 100°C in 'Bushel', 'Monodoro', and 'Break', respectively. More resistant cultivars showed a reduced INF compared to both less resistant and susceptible cultivars, of about 44% in 'Monodoro' and 79% in 'Break'. Furthermore, in 'Break' the LS was 45% smaller than that of the susceptible cultivar. Differences between cultivars were consistent from one experiment to another. Within cultivars variability was an important source of variation; it was attributed to both 'between plants' and 'between leaves within plants' variability. The former was due chiefly to the genetic heterogeneity of the cultivars used, which are open-pollened three-way cross hybrids; the latter, to differences in the age of the leaves of a plant.

RIASSUNTO

COMPONENTI DELLA RESISTENZA ALLA CERCOSPORIOSI IN BARBABIETOLA DA ZUCCHERO: DURATA DELL'INCUBAZIONE, EFFICIENZA DI INFEZIONE, DIMENSIONE DELLE LESIONI. Quattro varietà di barbabietola da zucchero con livelli crescenti di resistenza a *Cercospora beticola* ("Cyrano" o "Univers", "Bushel", "Monodoro" e "Break") sono state usate in esperimenti con singoli cicli di infezione, per studiare le seguenti componenti di resistenza: durata dell'incubazione (IP, sommatoria termica a base 5°C nel tempo fra l'inoculazione e la comparsa dei sintomi), efficienza di infezione dei conidi (INF, numero di macchie necrotiche per cm^2 di foglia al termine del ciclo infettivo) e dimensione delle lesioni (LS, area delle singole macchie). Tutte le componenti di resistenza sono state influenzate significativamente dalla varietà, mentre non sono state rilevate differenze nel tipo di lesioni. La resistenza ha ritardato la comparsa dei sintomi fino ad un massimo di 12 giorni rispetto alla varietà sensibile. L'IP_{50} è stato allungato di una sommatoria termica pari a 28°C, 60°C e 100°C in "Bushel", "Monodoro" e "Break", rispettivamente. Le varietà più resistenti hanno avuto una INF ridotta del 44% ("Monodoro") e del 79% ("Break") rispetto alle varietà sensibili o meno resistenti. Inoltre, "Break" ha mostrato macchie necrotiche più piccole del 45% rispetto alla varietà sensibile. Le differenze fra le varietà sono risultate costanti nei diversi esperimenti. La variabilità entro ciascuna varietà è apparsa una importante fonte di variazione. Ad essa hanno contribuito la variabilità fra piante, dovuta principalmente alla eterogeneità genetica delle varietà impiegate (che sono ibridi a 3 vie ad impollinazione aperta), e la variabilità fra foglie della stessa pianta, dovuta alla differente età delle foglie stesse.

Key words: Cercospora beticola, sugar beet, resistance components, incubation period, infection efficiency, lesion size.

INTRODUCTION

Cultivars of sugar beet resistant to Cercospora leaf spot (caused by *Cercospora beticola* Sacc.) are widespread in the Po Valley (northern Italy) and in other sugarbeet-growing areas (Rossi *et al.*, 1995).

It is known that Cercospora leaf spot resistance reduces the rate of progress of epidemics in the field

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(Rossi and Battilani, 1990; Rossi, 1995), according to Nelson's concept of rate-reducing resistance (Nelson, 1978). In epidemiological terms, resistance reduces the apparent infection rate as an effect of changes in the basic infection rate, due to a reduced effectiveness of each propagule in causing a new infection and a less abundant number of propagules produced per lesion per time unit (due to both reduced sporulation capability and reduced lesion size), in the latent period (that is the time between the arrival of a propagule on a susceptible plant surface and the formation of the next generation of propagules) and in the infectious period (that is the period of spore production on a lesion). Therefore, the components of resistance that reduce the rate of epidemic development are: infection frequency, latent period, lesion size, spore production, and infectious period (Parlevliet, 1979).

In some pathosystems involving Cercospora diseases the various components of rate-reducing resistance have been studied in depth. Resistance to *C. arachidicola* in peanuts has been tied in lesion density, lesion diameter, latent period, and amount of sporulation (Ricker *et al.*, 1985; Walls *et al.*, 1985; Waliyar *et al.*, 1993), as well as resistance to *C. zeae-maydis* in corn (Ringer and Grybauskas, 1995). Incubation period, lesion diameter, lesion number, and generation period have been shown to be important in resistance of soybean to *C. sojina* (Martins-Filho *et al.*, 1990).

A lot of work has been carried out to understand mechanisms of resistance to *C. beticola* in sugarbeet, acting both on the leaf surface (Pool and McKay, 1916; Kovacs, 1955; Solel and Minz, 1971; Ruppel, 1972; Feindt *et al.*, 1981; Whitney and Mann, 1981) and inside the leaf tissue after penetration (Brillova *et al.*, 1973; Geiger *et al.*, 1973; Hecker *et al.*, 1975; Brillova and Sladka, 1976; Johnson *et al.*, 1976; Steinkamp *et al.*, 1979; Feindt *et al.*, 1981; Lieber, 1982; Srobarova and Brillova, 1982; Nielsen *et al.*, 1994, 1997). Nevertheless, an organic sum of information about components of the rate-reducing resistance is not yet available.

The aim of this work is to study some resistance components involved in the rate-reducing resistance to *C. beticola* in sugar beet, and to quantify their effect. The study of mechanisms explaining resistance is not within the objectives of this work.

MATERIALS AND METHODS

In order to study the effect of host resistance on: (*i*) incubation period, (*ii*) infection efficiency of conidia and (*iii*) lesion size, we performed three experiments, using four sugar beet cultivars which exhibited different levels of rate-reducing resistance towards *C. beticola* infection in early screenings at the field level (Rossi, 1995), from susceptible ('Cyrano' or 'Univers'), to slightly ('Bushel'), moderately ('Monodoro'), and highly ('Break') resistant.

All the experiments were conducted in a greenhouse, by growing plants in big pots (1.4 x 2.2 m wide, 0.8 m high) on a natural substrate, under artificial light, with constant water supply. To simulate air temperature which usually occur during natural epidemics, we maintained a mean daily temperature of about 19°C in the first experiment, and 22°C in other experiments, with a daily range between about 16 and 27°C. The big pots had a transparent covering with two windows opening at a level which made it possible to control relative humidity accurately.

Sugar beet plants were transplanted into the big pots according to a completely randomized design, with eight replicates. In 1995 (Exp 1), we transplanted plants previously grown in a field at Fiorenzuola (North Italy), whose leaves had been previously cut leaving just the young central ones. In 1996 (Exp 2) and 1997 (Exp 3) we transplanted seed-borne plants, when they had two true leaves.

Plants were artificially inoculated with *C. beticola* about 8 weeks after transplanting; at this time they had 4 to 8 leaves near fully developed (no more increasing in size) and 6 to 10 leaves still growing, with different size according to their age. In Exp 1 we used a natural inoculum: naturally infected leaves of 'Cyrano' with typical leaf spot symptoms were cut, finely crushed, diluted in sterile water and shaken to obtain a suspension, which was filtered and adjusted to $3x10^5$ propagules per ml of water. In Exp 2 and 3 we used an *in vitro*-borne inoculum: three single-spore isolates collected earlier in Italy were grown on water agar (1.5% of agar) in Petri dishes and incubated at 20 ± 2°C for 2 weeks; colonies were then transferred onto Czapek-V8 agar (modified after Smith and Onions, 1983) and incubated at 24 ± 2°C for 1 week under UV lamps, 12 hours light/12 hours dark. Afterwards, the colonies were dispersed in distilled water; the resulting suspension was filtered, plated and incubated as previously described for 5 days. Finally, the surface of colonies was gently washed; the resulting suspension was filtered and adjusted to $3x10^5$ conidia per ml of water. The inoculum was obtained by mixing equal amounts of the spores produced by the three fungal strains.

The inoculum was applied with an atomizer over the plant canopy to cover the leaf surface uniformly; afterwards the plants were incubated at 95-98% relative humidity for 48 hours to favour infection. The plants were then grown for about 4 weeks at a relative humidity

lower than 60% to prevent sporulation.

The leaves were inspected daily for the appearance of necrotic spots, till spots finished appearing; the number of spots per leaf were recorded daily to avoid errors in counting due to coalescence. At the end of the experiment, both the total leaf area and the leaf area occupied by disease symptoms were measured (in cm^2) on each leaf. Leaf images were digitalized by a scanner (Hewlett Packard DeskScan II) and analysed by a home-made software previously elaborated and validated; it measures the number of pixel having different colours and transforms them in area units. Afterwards, the number of spots per cm^2 of leaf (spots number/total leaf area) and the size (in mm^2) of single necrotic spots (leaf area with symptoms·100/spots number) were calculated, the latter for the leaves where necrotic spots did not coalesce only.

In order to study variability within cultivars and within plants in incubation length, infection efficiency and lesion size we performed a fourth experiment, using 25 plants of two cultivars ('Univers' and 'Break'). Plant management, *C. beticola* inoculation, and measurements were carried out as previously described. For optimum management of the experiment, it was arranged in 5 sub-experiments, carried out at different times, each of these provided for 5 plants per cultivar and 4 leaves per plant.

To perform data analyses we considered three resistance components: incubation period (IP), infection efficiency of conidia (INF), lesion size (LS).

IP was expressed as the degree-days accumulated between inoculation and spot appearance on leaves. Degree-day cumulation was calculated by computing the mean of daily temperature (using 24 hourly values), using a base temperature of 5°C (Antonellini, 1974), as follows:

$$\sum_{t=1}^{n} T_t - 5$$

where: t=time in days from inoculation (t=1) to the end of the experiment (t=n); T_t=mean temperature of each t^{th} day (in °C); 5=base temperature (in °C).

INF was expressed as the number of necrotic spots per cm^2 of leaf at the end of the experiment, that is when spots stopped appearing, whereas LS was expressed as the area (in mm^2) of single spots at the end of the experiment.

The analysis of variance was performed on IP, INF, and LS measured on each leaf of the four cultivars in Exp 1 to 3. The experiment was considered as the 1st factor, with 3 levels, whereas the cultivar was considered as the 2nd factor, with 4 levels. Leaves were used as replicates, irrespective of the plant. Differences between means were tested using Tukey's Test at $P \leq 0.05$.

Since leaf spots did not appear on leaves simultaneously, the dynamic of IP over time was analysed by calculating the number of affected leaves on each day after inoculation. Since the number of leaves considered in each experiment was not the same, data were standardized as follows:

$$I_t = n_t / n_n$$

where: t=time in days from inoculation (t=1) to the end of the experiment (t=n); I_t=incidence of affected leaves on each t^{th} day; n_t=number of affected leaves on each t^{th} day.

The cumulative incidence of affected leaves (Y) was regressed on degree-day cumulation (X) by using the logistic model, which accurately fits disease progress over the time (Battilani and Rossi, 1988), in the following form:

$$Y = 1/[1 + \exp(a + b \cdot \ln(X))]$$

where: a and b are the parameters of the model. Analysis was performed by the nonlinear regression procedure of SPSS (SPSS Inc., 1995), which obtains least squares estimates of the parameters using the algorithm developed by Marquardt (1963). The goodness of fit was evaluated on the basis of the standard error of parameters, the R^2 statistic (calculated using the corrected total sum of squares), and distribution of residues versus predicted values. Degree-day cumulation at Y equal to 0.5 was then calculated as the degree-day cumulation when 50% of leaves get through the incubation period (IP_{50}).

Variability within cultivars and within plants in IP, INF, and LS was analysed by computing a hierarchical analysis of variance on data of Exp 4. The data set consisted of 3 response variables (degree-day cumulation for incubation, spots per cm^2 of leaf, area of single spots), each of these measured on each of the 100 leaves per cultivar: 4 leaves per plant, 5 plants per sub-experiment, 5 sub-experiments. Therefore the hierarchical classification was: cultivars, sub-experiments, plants within sub-experiments, leaves within plants. The variance component associated with each hierarchical group variable was calculated as a measure of the random effect of each variable. Then the proportion of variability in the response variable that was attributable to each group variable was calculated.

RESULTS

Incubation length. The first Cercospora spot constantly appeared on leaves of the susceptible 'Cyrano' or 'Univers', 4 (Exp 1), 8 (Exp 2) and 8 (Exp 3) days after inoculation, which corresponded to a degree-day cumu-

lation of 69.6, 166.1 and 140.5°C after inoculation (Table 1). The mean degree-day cumulation from inoculation was 125.4°C. Afterwards, spots continued appearing for 25 (Exp 1), 14 (Exp 2) and 18 (Exp 3) days after appearance of the first spot; when spots finished appearing the degree-day cumulation was 403.9, 389.2, and 395.7°C, respectively, with a mean of 396.3°C. In resistant cultivars the appearance of the first spots was delayed compared to the susceptible one by 5 days on average, with the exception of 'Monodoro' in Exp 1 and 'Bushel' in Exp 2, where the disease appeared at the same time as in the susceptible cultivar (Table 1). The maximum delay was 12 days for 'Break' in Exp 3. The mean degree-day cumulation for the first appearance of the disease was 178, 153 and 228°C for 'Bushel', 'Monodoro' and 'Break', respectively (Table 1).

Experiment and cultivar, as well as their interaction, influenced the incubation length significantly (Table 2). In Exp 2, IP (255.2°C on average) was shorter than in Exp 1 (279.8°C) and especially than in Exp 3 (312.8°C) (Table 3). IP increased significantly from the susceptible cultivar 'Cyrano' or 'Univers', to 'Bushel', 'Monodoro', and to the most resistant 'Break', changing from 241°C on average, to 269.1°C, 304.7°C and 338.9°C, respectively (Table 3). Differences between cultivars were not constant over experiments: 'Monodoro' and 'Break' did not differ significantly in Exp 1, nor did 'Cyrano' and 'Bushel' in Exp 2.

Table 1. Degree-day cumulation (in °C) between inoculation and the appearance of the first Cercospora leaf spot, in four sugar beet cultivars, in three experiments.

Experiment	Cultivar				Mean
	Cyrano/ Univers	Bushel	Monodoro	Break	
Exp 1	69.6	160.8	69.6	128.3	107.1
Exp 2	166.1	166.1	183.3	231.7	186.8
Exp 3	140.5	205.5	205.5	324.6	219.0
Mean	125.4	177.5	152.8	228.2	171.0

Table 2. Analysis of variance for three resistance components to Cercospora leaf spot, in four sugar beet cultivars.

Source of variation	Incubation length			Infection efficiency			Lesion size		
	d.f.	F value		d.f.	F value		d.f.	F value	
Experiment	2	18.33	**	2	1.95	ns	2	13.97	**
Cultivar	3	31.41	**	3	13.42	**	3	2.94	*
Cultivar x experiment	6	6.86	**	6	1.11	ns	6	0.64	ns
Residual	389			491			142		
Total	400			502			153		

Ns: not significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$.

Table 3. Incubation length of Cercospora leaf spot, in four sugar beet cultivars, in three experiments (expressed as degree-day cumulation, in °C).

Experiment	Cultivar				Mean
	Cyrano/ Univers	Bushel	Monodoro	Break	
Exp 1	257.1	272.6	300.2	290.8	279.8 B
Exp 2	221.8	225.3	260.0	318.1	255.2 C
Exp 3	226.7	295.7	361.9	432.3	312.8 A
Mean	241.0	269.1	304.7	338.9	
	D	C	B	A	

Means followed by different letters are significantly different according to Tukey's Test with $P \leq 0.05$.

Cultivars differed from each other not only in mean IP, but in the distribution of affected leaves over the time after inoculation too. Incidence of affected leaves in the susceptible cultivar increased faster than in the other cultivars (Table 4). Pooling data from Exp 1 to 3, distribution of affected leaves in 'Bushel', 'Monodoro' and 'Break' differed significantly from distribution in 'Cyrano' or 'Univers'.

In both susceptible and resistant cultivars the incidence of affected leaves progressed following an S-shaped trend. The fitting of data by the logistic function gave satisfactory results in both single and pooled experiments (Table 5): the standard error of parameters was constantly low, the R^2 was greater than 0.90, and distribution of residues was always homogeneous over the whole range of the independent variable (not shown).

The IP_{50} calculated by the fitted functions was 209.5°C for the susceptible cultivar, 237.5°C for 'Bushel', 269.7°C for 'Monodoro', and 309.3°C for 'Break' (Fig. 1).

Infection efficiency of conidia. All the disease symptoms observed were typical Cercospora leaf spots: small, nearly circular, clearly separated from healthy tissue, light brown to grey or whitish spots appeared on the leaves of any cultivar; no brown or reddish purple borders were observed.

Each infection produced many spots on the leaves; about 58,000 spots were counted in all during Exp 1 to 3. The maximum number was 2120 spots on a leaf of 'Univers', whose mean was 269. The mean number of spots per leaf was 134 (max 661) for 'Bushel', 93 (max 574) for 'Monodoro', and 40 (max 263) for 'Break'.

The number of spots per cm^2 of leaf was significantly influenced by cultivar; on the contrary, experiment and cultivar x experiment interaction did not influence it significantly (Table 2). The susceptible cultivar and 'Bushel' did not differ from each other significantly; they showed 1.33 and 1.05 spots per cm^2 of leaf on average, respectively. 'Monodoro' and 'Break' showed fewer spots on leaves, the former having 0.57 spots, the latter 0.35 (Fig. 2).

Frequency of healthy leaves strongly influenced the previous results (Fig. 3). 40.7% of 'Break' leaves did not show any symptoms though inoculated, besides 57.1% of leaves had fewer than 2 spots per cm^2. In contrast, only 7.8% of leaves of the suceptible cultivar did not show symptoms, furthermore 22.4% of leaves had more than 2 spots per cm^2, up to 8 spots. 'Bushel' and 'Monodoro' showed the same frequency of healthy leaves (19.7% and 20.1%, respectively), but the former had a greater number of leaves with more than 2 spots per cm^2, up to 6 spots (18.3%), and some leaves with more than 8 spots.

Table 4. Incidence of leaves affected by Cercospora leaf spot at several times after inoculation, in four sugar beet cultivars.

	Days after inoculation									
Cultivar	1	4	7	10	13	16	19	22	25	28
Exp 1										
Cyrano	0.034	0.080	0.126	0.207	0.575	0.655	0.816	0.920	1.000	
Bushel	0.000	0.000	0.028	0.153	0.541	0.708	0.820	0.903	1.000	
Mondoro	0.015	0.030	0.045	0.061	0.363	0.591	0.863	0.910	1.000	
Break	0.000	0.000	0.150	0.150	0.201	0.250	0.799	0.901	1.000	
Exp 2										
Cyrano	0.030	0.640	0.960	0.960	0.960	1.000				
Bushel	0.010	0.570	0.952	0.952	0.952	1.000				
Mondoro	0.000	0.200	0.800	0.850	0.900	1.000				
Break	0.000	0.000	0.381	0.667	0.810	0.905	1.000			
Exp 3										
Univers	0.036	0.143	0.821	0.893	0.964	0.964	1.000			
Bushel	0.000	0.000	0.160	0.480	0.800	0.960	1.000			
Mondoro	0.000	0.000	0.043	0.261	0.565	0.696	0.739	0.783	0.913	1.000
Break	0.000	0.000	0.000	0.000	0.333	0.500	0.667	0.667	0.750	1.000

Table 5. Parameters and statistics of the logistic equations fitting the incidence of leaves affected by Cercospora leaf spot over the degree-days accumulated from inoculation, in four sugar beet cultivars, in three experiments.

Cultivar	a	S.E. a	b	S.E. b	R^2
Exp 1					
Cyrano	43.22	0.830	-7.82	0.155	0.96
Bushel	41.79	0.498	-7.54	0.093	0.99
Monodoro	40.15	0.419	-7.08	0.075	0.99
Break	38.92	1.222	-6.85	0.220	0.89
Exp 2					
Cyrano	90.31	0.488	-16.91	0.093	0.99
Bushel	71.53	0.384	-13.32	0.074	0.99
Monodoro	46.28	0.465	-8.46	0.087	0.99
Break	45.87	0.534	-8.06	0.097	0.98
Exp 3					
Univers	71.66	0.786	-13.86	0.155	0.99
Bushel	51.82	0.529	-9.37	0.096	0.99
Monodoro	53.85	1.016	-9.45	0.181	0.96
Break	48.97	1.077	-8.33	0.186	0.91
Pooled					
Cyrano/Univers	50.49	0.881	-9.45	0.169	0.91
Bushel	46.44	0.526	-8.49	0.098	0.96
Monodoro	45.54	0.493	-8.14	0.090	0.95
Break	46.10	0.570	-8.04	0.106	0.91

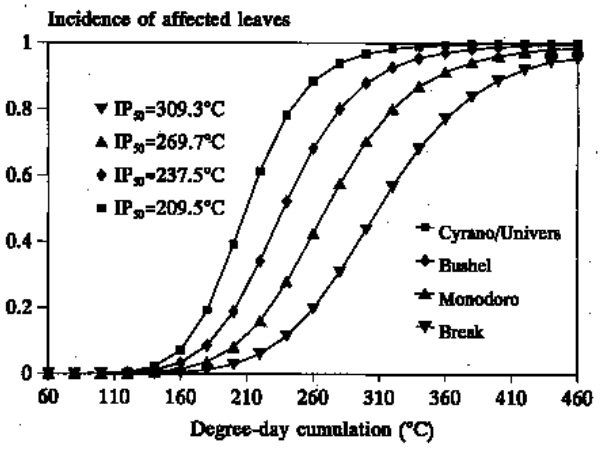

Fig. 1. Incidence of leaves affected by Cercospora leaf spot in four sugar beet cultivars, on different degree-days accumulated from inoculation. Values were calculated by means of logistic equations whose parameters are shown in Table 5 (pooled data from Exp 1 to 3). IP_{50} is the degree-day cumulation when 50% of leaves have been affected.

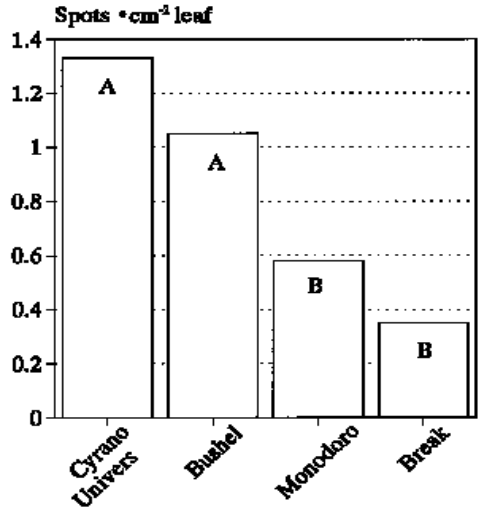

Fig. 2. Number of Cercospora leaf spots per cm^2 of leaf in four sugar beet cultivars. Bars represent means of Exp 1 to 3; bars with different letters are significantly different according to Tukey's Test with $P \leq 0.05$.

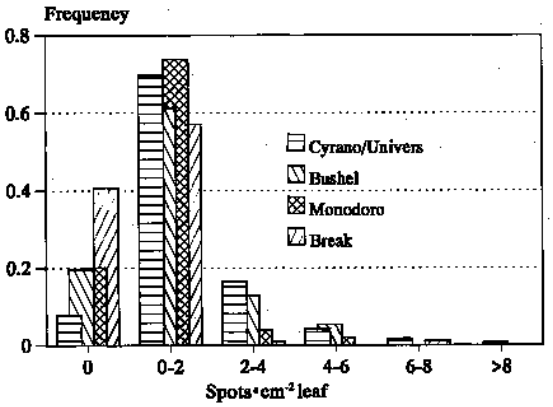

Fig. 3. Frequency distribution of the leaves of four sugar beet cultivars in classes with different numbers of Cercospora spots per cm^2 of leaf (pooled data from Exp 1 to 3).

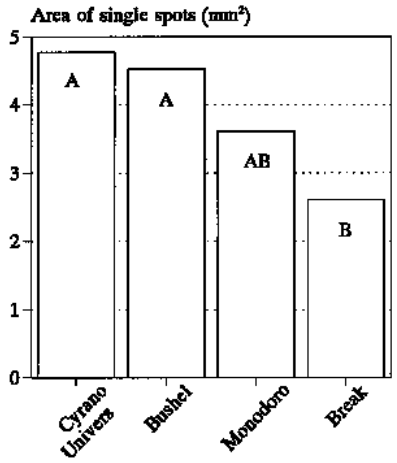

Fig. 4. Area (mm^2) of single Cercospora leaf spots in four sugar beet cultivars. Bars represent means of Exp 1 to 3; bars with different letters are significantly different according to Tukey's Test with $P \leq 0.05$.

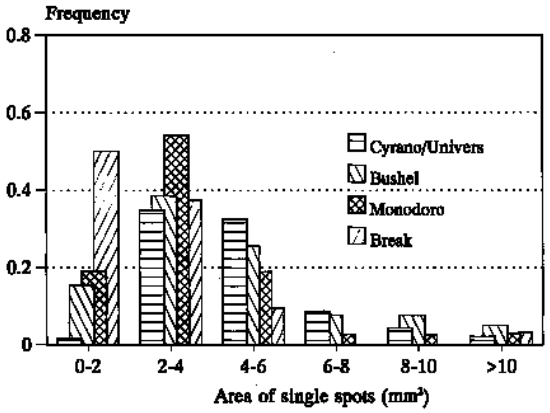

Fig. 5. Frequency distribution of the leaves of four sugar beet cultivars in classes with different areas (mm^2) of single Cercospora spots (pooled data from Exp 1 to 3).

Lesion size. Both experiment and cultivar influenced LS significantly, whereas their interaction did not show a significant effect (Table 2). Differences between experiments accounted for a great part of variability: Exp 2 and 3 did not differ from each other, but they had spots about 1/3 wider than the spots of Exp 1. 'Break' had the smallest spots, which measured 2.6 mm^2 each on average. They were different from the spots of both the susceptible cultivar and 'Bushel', which were 4.8 and 4.5 mm^2 wide, respectively (Fig. 4). The spots of 'Monodoro' did not differ from either 'Break' or the other cultivars significantly, being 3.6 mm^2 wide.

In 'Break', spots less than or equal to 2 mm^2 were the most frequent (50%) and no spots had an area greater than 6 mm^2, with the exception of one leaf where spots were 11 mm^2 wide. In other cultivars, the most common class was that with spots 2-4 mm^2 wide, even if in the susceptible cultivar spots of 4-6 mm^2 were almost equally frequent (Fig. 5).

Intra-cultivar variability in resistance components. Variability in IP, INF, and LS within the 100 leaves considered in Exp 4 was wide for both 'Univers' and 'Break'. Incubation length showed the lowest variability among the resistance components: IP ranged between 159.1°C and 483.8°C for 'Univers' (mean=266.5°C; s.d.=73.18°C), whereas for 'Break' it lay between 173.4°C and 496.3°C (mean=333.9°C; s.d.=83.77°C). In contrast, infection efficiency of conidia showed the highest variability: the minimum INF was equal to zero for both cultivars, whereas the maximun was 6.5 spots per cm^2 of leaf for 'Univers' (mean=0.6; s.d.=1.11), and 3.0 spots for 'Break' (mean=0.2; s.d.=0.44). Lesion size ranged between 1 and 11.2 mm^2 for 'Univers' (mean=6.0; s.d.=4.33), and between 0.4 and 7.4 mm^2 for 'Break' (mean=3.2; s.d.=2.16).

Hierarchical analysis of variance (Table 6) showed that variability between sub-experiments accounted for a small part of variance (5.5 to 9.9% of variance component) for all the resistance components. Variability between plants within sub-experiments accounted for a greater part of variance compared to variability between sub-experiments (14.1 to 23.4%), especially for IP, whereas variability between leaves within plants was the greatest source of variation (43 to 62.2%).

DISCUSSION

The incubation length we observed was in general agreement with other authors (Wallin and Loonan, 1971; Dumitras, 1975; Rathaiah, 1977), who found 5 to

Table 6. Percent variance component associated with each source of variation resulting from the hierarchical analysis of variance for three resistance components to Cercospora leaf spot, in two sugar beet cultivars ('Univers' and 'Break').

		Incubation length		Infection efficiency		Lesion size	
Source of variation	d.f.	F value	%	F value	%	F value	%
Between cultivars	1	8.1 *	23.71	5.4 *	11.22	11.0 *	22.48
Between sub-experiments within cultivars	8	2.5 *	9.89	2.1 ns	7.83	2.0 ns	5.51
Between plants within sub-experiments	40	3.2 **	23.37	2.2 **	18.76	2.0 **	14.11
Between leaves within plants	150		43.02		62.19		57.90
Total	199						

Ns: not significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$.

10 days of incubation under changing environmental conditions. At field level in the Po Valley (Italy) it was 19 to 21 days at 15°C, 9 to 10 days at 25°C, and 7 to 8 days at 30°C (Canova, 1959b). In our work, IP of the susceptible cultivar ranged between 4 and 9 days, depending on the experiment. The cumulation of daily temperatures occurred since inoculation till spot appearance showed that the mean degree-day cumulation for the appearance of the first Cercospora spot was of 153°C, with a 95% confidence interval between 128 and 177°C. Afterwards spots continued appearing over a 2-4 week period, that is a mean degree-day cumulation of 280°C (between 235 and 324°C): IP_{50}, that is the degree-day cumulation when 50% of leaves get through the incubation period, was 210°C. These values agreed with findings of Bleiholder and Weltzien (1971), Antonellini (1974) and Mischke (1980).

In resistant cultivars the appearance of the first necrotic spots was delayed compared to the susceptible one by 5 days on average, to a maximum of 12 days; IP_{50} was equal to 209.5°C in the susceptible cultivar and it was delayed by a degree-day cumulation of 28°C, 60°C and 100°C in 'Bushel', 'Monodoro' and 'Break', respectively; therefore, the effect of resistance in increasing the incubation length compared to the susceptible cultivar was 12%, 26% and 41%, for 'Bushel', 'Monodoro' and 'Break', respectively. Cultivars differed from each other significantly.

The resistance level influenced the infection efficiency of conidia significantly: the more resistant 'Break' and 'Monodoro' showed a reduced number of spots per cm^2 of leaf compared to both the less resistant 'Bushel' and the susceptible 'Cyrano' or 'Univers', by about 26%, 44% and 79%, respectively.

Following a similar approach, Solel and Wahl (1971) graded reaction of sugar beet cultivars by relating the density of leaf spots induced by artificial inoculation to that of a susceptible standard, as follows: susceptible, more than 80%; moderately susceptible, 50-80%; moderately resistant, 30-50%; resistant, less than 30%.

Solel and Minz (1971) explained the reduced number of spots on leaves of two resistant cultivars by histological studies. Since they found that any fungal development ceased 16 days after inoculation (at 25°C), they attributed the ability of a cultivar to overcome hyphal colonization of leaf tissue till the development of necrotic phase to the time interval between penetration of germ tubes and the arresting phase. In fact, in their study only 48% of the germ tubes penetrating the leaves of the most resistant cultivar developed to the necrotic phase, whereas 79% of penetrations caused necrosis in the susceptible test.

In all the cultivars we observed the same type of reaction to *C. beticola* inoculation, typical the necrotic spots. Similar results were obtained by Solel and Wahl (1971), whereas Whitney and Mann (1981) found two more types by inoculating Race C2 on the cultivar FC 701/2: a large chlorotic fleck and a small chlorotic fleck; they considered the typical spot to be a susceptible reaction. A fleck reaction was observed by Carels *et al.* (1990) too, in both *Beta webbiana* and *B. procumbens*.

The size of the spots we measured were wider than those observed by Whitney and Mann (1981), but it is whithin the limits found by Solel and Wahl (1971). We found a significant difference between cultivars in the area of single spots. In particular, 'Break' had a 45% smaller lesion size than the susceptible cultivar.

Differences between cultivars we observed in the resistance components can be considered consistent from one experiment to another. In fact, variability between experiments was found to be a significant source of variation only when the IP and LS found in Exp 1 were

compared to those from Exp 2 and 3. With the aim of annulling the effect of some factors influencing the incubation period length (Wallin and Loonan, 1971), we maintained constant spore concentration and length of wet periods over experiments, and we controlled temperature inside each experiment. Notwithstanding this, Exp 1 differed from all the other experiments because we used a natural inoculum of *C. beticola* instead of an *in vitro*-borne inoculum. Solel and Minz (1971) observed different penetrations throughout stomata using a field collected inoculum (96%) and an *in vitro*-borne inoculum (83%). In the natural inoculum, several conidial developmental stages were certainly present, from newly developed conidia to fully developed ones, the latter having both a higher germination rate and a greater germ tube growth than to the former (Canova, 1959a). Variability in spore development inside the natural inoculum could account for differences in IP we observed in Exp 1. However, the difference in LS between Exp 1 and the other experiments could be attributed to temperature, which was lower in Exp 1 than in the other cases. In fact, Waliyar *et al.* (1993) showed that the lesion diameter of Cercospora leaf spot on peanuts depends on temperature as well as on genotype.

Within cultivar variability was an important source of variation. Between plants variability can be attributed to both environment and the genetic heterogeneity of sugar beet cultivars, which are open-pollened three-way cross hybrids. Differences between plants in our work were of the same magnitude of variability found by Smith and Gaskill (1970) and De Biaggi and Rubboli (1984); the former therefore calculated variance for resistance of 50 *in vitro*-borne clones, the latter considered inbred lines presumably highly homozygous in resistance or susceptibility. In such cases, the plant to plant variation within each clone or inbred was considered environmental. Because our environmental conditions were more constant than those of the previously cited works, which were carried out at field level, genetic heterogeneity of cultivars probably played an important role in our results.

Variability between leaves within plants can be caused chiefly by differences in age of leaves. It has been demonstrated that susceptibility to infection of leaves increases from young, to old, to mature fully expanded leaves (Canova, 1959b; Rossi *et al.*, 1990). Feindt *et al.* (1981) observed that the effect of leaf age on the degree of infection was so evident that disease severity was similar on younger leaves of resistant plants and older ones of susceptible cultivars. The mechanism of resistance to infection in young sugar beet leaves has been widely studied (Pool and McKay, 1916; Canova, 1959b), but it is still unexplained (Ruppel, 1972). More recently, Singh *et al.* (1986) showed that *C. beticola* growth and germination were inhibited by exudates of young leaves of spinach.

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