SUMMARY

Four sugar beet cultivars exhibiting increasing levels of rate-reducing resistance towards Cercospora beticola (‘Cyrano’, ‘Bushel’, ‘Monodoro’, and ‘Break’) were used to study two resistance components: length of conidiation period (CP, the time required to produce conidia on the spots incubated under optimal conditions for sporulation) and spore yield (SY, the number of conidia yielded per cm² of necrotic area per time unit); morphology and germination of conidia produced on the different cultivars were also considered. Experiments were carried out over a three-year period, considering sporulation on naturally infected leaves, either under field conditions or under optimal laboratory conditions, and on artificially inoculated leaves. Level of host resistance did not influence CP significantly: all spots produced conidiophores bearing conidia extensively 3 days after they had been stimulated to sporulate. On the contrary, resistance affected SY: the more resistant ‘Monodoro’ and ‘Break’ yielded fewer conidia per unit of necrotic area than the susceptible ‘Cyrano’ and the less resistant ‘Bushel’; the former cultivars produced about 35% of the conidia yielded by the latter ones. Differences in the cultivar rating were consistent over the experiments. Spores produced on the susceptible ‘Cyrano’ were longer, wider, and had more septa than the conidia yielded on the resistant cultivars, irrespective of their resistance level; they germinated more quickly than conidia yielded on other cultivars, and germ tubes grew more rapidly.

Key words: Cercospora beticola, sugar beet, resistance components, conidiation period, spore yield.

INTRODUCTION

Resistant cultivars play an important role in the control of Cercospora leaf spot epidemics (caused by Cercospora beticola Sacc.) on sugar beet (Rossi et al., 1995), because they reduce the rate of disease progress in the field (Rossi, 1995), according to Nelson’s concept of rate-reducing resistance (Nelson, 1978).

In a previous work (Rossi et al., 1998), four sugar beet cultivars exhibiting different levels of rate-reducing resistance towards C. beticola, from susceptible to highly resistant, showed significant differences in: incubation length, infection efficiency of conidia, and lesion size. The incubation period was lengthened by 12%, 26%, and 41%, in slightly, moderately, and highly resistant cultivars, respectively, when compared with a susceptible cultivar. Infection efficiency was reduced significantly by 44% and 79% in the two more resistant hosts, whereas lesion size was reduced by 45% in the most resistant one only.

In other pathosystems involving Cercospora diseases, sporulation has been shown to play an important role in rate-reducing resistance, together with other resistance components like incubation, lesion density, and lesion diameter (Ricker et al., 1985; Walls et al., 1985; Martins-Filho et al., 1990; Waliyar et al., 1993; Ringer and Grybauskas, 1995). In such cases, both the latent period and spore production have been considered, the former being the time elapsing between the arrival of a spore on the plant surface and the formation of the next generation of spores, the latter being the total amount of spores yielded per lesion, or per unit area of affected tissue, per unit of time (Parlevliet, 1979). Sufficient information is not available on the role of these resistance components in the C. beticola-sugarbeet pathosystem.

C. beticola produces conidia on the surface of necrotic spots, which appear on leaves after the incubation period; when environmental conditions are favourable (Canova, 1959c), conidiophores bearing conidia project above the lesion surface, extending from darkly pigmented spots (pseudostromata) scattered throughout the centre of the lesions (Steinkamp
et al., 1979). Since sporulation of the fungus does not occur until the leaf tissue has become necrotic (Whitney and Mann, 1981), the whole latent period can be divided into incubation (the time between inoculation and first visible symptoms) and conidiation (the time between appearance of spots and spore production) (Rossi et al., 1994).

In order to complete previous studies (Rossi et al., 1998) on the components of rate-reducing resistance to Cercospora leaf spot in sugar beet, some experiments were carried out in which three cultivars exhibiting different resistance ratings were compared to a susceptible cultivar as regards the length of conidiation period, the spore yield, the morphology and germination of conidia.

MATERIALS AND METHODS

Several experiments were carried out in order to study: (i) conidiation length, (ii) spore yield on necrotic spots; (iii) morphology and (iv) germination of the conidia yielded on spots. The same cultivars used in a previous work (Rossi et al., 1998) were selected for this study. They exhibited different levels of rate-reducing resistance towards C. beticola infection in early screenings at the field level (Rossi, 1995), from susceptible (‘Cyrano’), to slightly (‘Bushel’), moderately (‘Monodoro’), and highly (‘Break’) resistant.

In 1994, the 4 cultivars were grown in big plots (200 m² each) at Fiorenzuola (North Italy) under conditions of natural C. beticola infections. Ordinary agricultural techniques were applied, without any fungicidal spray. On 16 occasions, between August 1 and September 29, 2 leaf disks with typical non-coalescing Cercospora spots were cut, with a 1.08 cm² punch, from 10 randomly collected fully developed leaves per cultivar. Leaf disks were carefully maintained at 5°C and carried to the laboratory. The leaf area (in cm²) covered by necrotic spots was measured on each disk by a homemade software previously elaborated and validated, which uses the images digitalized by a scanner (Helwett Packard DeskScan II). Afterwards, the disks were finely crushed by a cutter, placed in test-tubes (4 disks per tube) with 0.3 ml sterile water, and shaken for 1 min to obtain a spore suspension. Three 10 µl drops were drawn from each tube and placed on a microscope slide. The number of conidia visible under a microscope (20 x) were counted in 10 fields for each drop. Therefore the total number of the conidia per cultivar per survey was calculated by means of the useful transformation (based on both the microscope field width and dilution) on the basis of conidia recorded on 30 fields for each of the 5 replicates (5 tubes, 4 leaf disks each). Finally, the spore yield was expressed as the number of conidia per cm² of necrotic area (total number of conidia/necrotic area).

In 1995, the 4 cultivars were grown as previously described, at Fiorenzuola. On September 5 and 12, 20 leaves naturally infected by C. beticola were collected from each plot: fully expanded leaves rating 10-20% of disease severity, with typical non-coalescing symptoms were chosen. Leaves were cut and carried to the laboratory, where 75 leaf disks were cut from leaves of each cultivar as previously described. The leaf disks were washed under running tap water for 15 min to remove conidia which had been previously differentiated. Afterwards, the disks were dried at room temperature and the leaf area occupied by necrotic spots measured. The disks were then laid on microscope slides and placed in Petri dishes (3 disks per dish), containing wet filter-paper to provide 100% relative humidity. The dishes were closed with a transparent plastic film, and incubated at 25±2°C, in daylight (12 hours dark/12 hours light). In all, 20 dishes per cultivar were prepared. After 6, 12, 24, and 48 hours incubation, 5 dishes per cultivar were opened and the leaf disks treated as previously described, to calculate the number of conidia per cm² of necrotic area for each of the 4 cultivars, in each of the 4 incubation times, for each of the 5 replicates (5 test-tubes, 3 leaf disks each). A part of the suspension of conidia yielded on necrotic spots after 24 hours incubation was observed under the microscope to measure both the length and width of the conidia (µm) and count the number of septa; observations were made on a total of 450 conidia per cultivar. Drops of the spore suspension were placed on microscope slides in Petri dishes, containing wet filter-paper. The dishes were closed with a transparent plastic film, and incubated at 25±2°C, in daylight (12 hours dark/12 hours light). After 1, 1.5, 3, and 6 hours incubation, the drops were observed under the microscope to determine: (i) the number of conidia germinating (conidia were considered to have germinated when a germ tube was produced, irrespective of its length); (ii) length of the germ tube developed from the basal cell of the conidium (in µm). Observations were made on 150 conidia per cultivar.

In 1996, plants from seeds of the 4 cultivars were germinated in pots and then transplanted in larger pots (1.4 x 2.2 m wide, 0.8 m high) when they had two true leaves, according to a completely randomized design, with eight replicates. The experiment was carried out in a greenhouse, growing the plants under artificial light, at a temperature of about 22°C, with constant water supply (see Rossi et al., 1998, for further details). Plants
were artificially inoculated 8 weeks after transplanting using an in vitro-culture of C. beticola, which was prepared as described in a previous paper (Rossi et al., 1998). The inoculum (3 x 10⁵ conidia per ml of water) 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. Plants were then grown for about 4 weeks at a relative humidity lower than 60% to prevent sporulation. Leaves were inspected daily for the appearance of necrotic spots, until this no longer occurred. In the first experiment, 64 leaf disks with non-coalescing spots were removed from the leaves (2 disks per leaf, 4 leaves per each of the 8 plants) on the day when spots appeared and for 3, 5, and 7 days after: four groups of spots were then made, each of spots of a different age. The disks were incubated, as previously described, for 72 hours. Afterwards the spots were observed under a stereomicroscope (40 x) and classified on the basis of presence or absence of sporulation: spots were considered sporulated when clusters of conidiophores with developed conidia were visible. In the second experiment, 64 leaf disks with single spots were cut, irrespective of the age of spot, and processed as previously described. The spots were microscopically observed after 16, 48, 64, 90 and 114 hours incubation to note the presence or the absence of sporulation. Both experiments were repeated twice. When spots finished appearing, 6 leaf disks were cut from the leaves, treated as described in the experiment carried out in 1995, and incubated for 24 hours. Afterwards, the number of conidia per cm² of necrotic area for each of the 4 cultivars was determined. To perform data analyses we considered: (i) length of conidiation period (CP), (ii) spore yield on necrotic spots (SY). Morphology of conidia yielded on spots was analysed by: (i) length of conidia (CL), (ii) width of conidia (CW), and (iii) number of septa per conidium (SN), whereas germination was analysed by: (i) proportion of germinated conidia (GC), (ii) length of germ tubes (GTL).

CP was expressed as the time required to produce conidia on the spots incubated under optimal conditions for sporulation. The proportion of sporulating spots in each of the 4 cultivars, at each spot age and on each incubation time, was calculated on the data collected in 1996, during the first and the second experiment, respectively. To test the null hypothesis that the proportion of sporulating spots has the same mean (H₀: µ₁ = µ₂), versus the alternative hypothesis H₁: µ₁ ≠ µ₂, the test F was previously applied to verify that populations have the same variance.

An AOV for a two factor (cultivar, 4 levels; incubation time, 4 levels) randomized design combined over days (random variable, 2 levels), with 5 replicates was performed on the data of GC and GTL recorded in 1995. GC data, which are proportions, were previously transformed [arcsin(√x)]. Differences between means were tested performing Tukey’s Test at P ≤ 0.05 on transformed data. Afterwards, data were back-transformed.

RESULTS

Conidiation length. The X² Test showed that neither cultivar nor leaf spot age significantly influenced the time required for sporulation. In fact, about 100% of 1 day-old spots as well as 7 day-old spots were able to produce both conidiophores and conidia extensively after 72 hours of incubation under optimal conditions for sporulation, irrespective of cultivar (data not shown). Likewise, cultivars did not differ from each other significantly regarding the proportion of sporulating spots over the time when spots were stimulated to sporulate (Fig. 1). Irrespective of the cultivars, all the sporulated spots showed a greyish-white necrotic centre, with many scattered, small, darkly pigmented pseudostromata, from which conidiophores bearing conidia extended.
Spore yield on necrotic spots. In 1994, conidia produced in vivo on spots collected from naturally infected leaves were significantly (P < 0.01) influenced by cultivar: on an average of the 16 surveys, ‘Cyrano’ and ‘Bushel’ produced more conidia than ‘Monodoro’ and ‘Break’ (Table 1). Similar results were obtained in 1995 and 1996: conidia yielded in vitro, after 24 hours incubation, on spots collected from naturally infected leaves and on artificially inoculated leaves, respectively, were significantly (P < 0.01) affected by cultivar. In both experiments, the SY was significantly greater for the spots collected from leaves of the susceptible ‘Cyrano’ or the less resistant ‘Bushel’ than those from the leaves of the more resistant ‘Monodoro’ or ‘Break’ (Table 1).

Field experiments made in 1994 showed that differences between cultivars were consistent over the time of epidemic. In fact, cultivars rated as previously described in 12 surveys out of 16; in the 4 remaining cases, differences between cultivars were not significant because no or very low amount of spores were produced, due to unfavourable environmental conditions. Laboratory experiments carried out in 1995 demonstrated that differences between cultivars were also consistent over the period of incubation under optimal conditions for sporulation. In fact, interaction between cultivar and incubation time did not influence SY significantly (Fig. 2).

Morphology of conidia produced on spots. According to the t Test, populations of conidia produced in vitro on spots collected in 1995 from naturally infected leaves of ‘Cyrano’ differed (with P < 0.01) from those of other cultivars significantly, in contrast, the other cultivars did not significantly differ from one another. Conidia produced on the susceptible cultivar measured 129.5 µm length, 2.7 µm width, and had 20.6 septa on average. Conidia yielded by ‘Bushel’, ‘Monodoro’, and ‘Break’ measured less both in length and width, and had fewer septa (Table 2).

### Table 1. Number of C. beticola conidia produced per cm² of necrotic leaf area, in three experiments carried out under different conditions.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>In vivo on naturally infected leaves 1994</th>
<th>In vitro on naturally infected leaves ¹ 1995</th>
<th>In vitro on artificially inoculated leaves ¹ 1996</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyrano</td>
<td>3135 A</td>
<td>6576 A</td>
<td>5992 A</td>
<td>5234</td>
</tr>
<tr>
<td>Bushel</td>
<td>2783 A</td>
<td>4160 A</td>
<td>4001 A</td>
<td>3648</td>
</tr>
<tr>
<td>Monodoro</td>
<td>2108 B</td>
<td>1319 B</td>
<td>1690 B</td>
<td>1706</td>
</tr>
<tr>
<td>Break</td>
<td>1920 B</td>
<td>2254 B</td>
<td>2011 B</td>
<td>2062</td>
</tr>
<tr>
<td>Mean</td>
<td>2487</td>
<td>3577</td>
<td>3424</td>
<td></td>
</tr>
</tbody>
</table>

Letters show signifiycativity of the difference between values inside each experiment, according to the Tukey’s Test at P ≤ 0.05; the test was performed on previously transformed data [ln(x+1)], then data were back-transformed [antilog(x)-1] to be tabulated.

¹ After 24 hours incubation under optimal conditions for sporulation.
Germination of conidia produced on spots. Germination of conidia yielded in vitro on spots collected from naturally infected leaves of four sugar beet cultivars, and incubated for several times. Letters in the legend show significativity of the differences between cultivars, according to Tukey’s Test at $P \leq 0.05$; because of no significant interaction ‘cultivar x incubation time’, significativity of differences is constant over the hours of incubation.

### Table 2. Morphology of *C. beticola* conidia produced in vitro on necrotic spots collected from naturally infected leaves of four sugar beet cultivars, in 1995.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Morphological features</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length (µm)</td>
<td>Width (µm)</td>
<td>Septa (number)</td>
<td></td>
</tr>
<tr>
<td>Cyrano</td>
<td>129.5 ± 3.58$^{1}$</td>
<td>2.7 ± 0.06</td>
<td>20.6 ± 0.71</td>
<td></td>
</tr>
<tr>
<td>Bushel</td>
<td>112.1 ± 3.11</td>
<td>2.4 ± 0.05</td>
<td>17.6 ± 0.60</td>
<td></td>
</tr>
<tr>
<td>Monodoro</td>
<td>119.2 ± 3.37</td>
<td>2.5 ± 0.06</td>
<td>18.6 ± 0.67</td>
<td></td>
</tr>
<tr>
<td>Break</td>
<td>117.2 ± 3.41</td>
<td>2.5 ± 0.06</td>
<td>18.2 ± 0.68</td>
<td></td>
</tr>
</tbody>
</table>

$^{1}$ Mean and standard error of 450 conidia.

**Fig. 2.** Number of *C. beticola* conidia produced in vitro on necrotic spots collected in 1995 from naturally infected leaves of four sugar beet cultivars, and incubated for several times. Letters in the legend show significativity of the differences between cultivars, according to Tukey’s Test at $P \leq 0.05$; because of no significant interaction ‘cultivar x incubation time’, significativity of differences is constant over the hours of incubation.

**Fig. 3.** Germination (%) of *C. beticola* conidia produced in vitro on necrotic spots collected from naturally infected leaves of four sugar beet cultivars, and incubated for different times. Letters show significativity of the differences between cultivars on each incubation time, according to Tukey’s Test at $P \leq 0.05$; no letters means no significant differences.

**Fig. 4.** Length of germ tubes grown from *C. beticola* conidia produced in vitro on necrotic spots collected from naturally infected leaves of four sugar beet cultivars, and incubated for different times. Letters in the legend show significativity of the differences between cultivars, according to Tukey’s Test at $P \leq 0.05$; because of no significant interaction ‘cultivar x incubation time’, significativity of differences is constant over the hours of incubation.
DISCUSSION

Conidiation length on Cercospora leaf spots observed in the present work was in agreement with previous findings. Steinkamp et al. (1979) obtained sporulation after 3 days, when leaf lesions were incubated at 100% relative humidity and 30°C. Whitney and Mann (1981) found that the mean time required to obtain sporulation on 100% of typical Cercospora spots was 3.1 days, when spots were incubated at 15°C. Canova (1959c) observed that the formation of conidia directly on the host under natural conditions in the Po Valley (North Italy) occurred after a minimum of 3-4 days. In the present experiments, all spots produced conidiophores bearing conidia extensively 3 days after they had been stimulated to sporulate, irrespective of the age of the spots. The level of host resistance did not influence conidiation length significantly. We did not find in the literature any previous results on the time for C. beticola conidiation in sugar beet cultivars exhibiting rate-reducing resistance. Solel and Wahl (1971) reported that lesions produced on many resistant sugar beet entries showed similar copious sporulation, but no more information has been given on the meaning of copious sporulation. Whitney and Mann (1981) observed that sugar beet plants showing a resistant reaction to race C2 required a longer time for sporulation than plants having a susceptible reaction. In the cited work, the resistant reaction referred to the lesion type, which showed flecks instead of necrotic spots, whereas in the present work spots from cultivars all showing the typical Cercospora leaf spots were considered (Rossi et al., 1998).

Spore yield found in the present work was of about the same magnitude as the sporulation capacity observed by Canova (1959c), but less than that calculated by Frandsen (1956). Spore yield per unit of necrotic area was consistent over the experiments (Table 1), though different methods were applied. In 1994, the number of conidia yielded per spot under natural conditions, in the field, during several days of a Cercospora leaf spot epidemic were counted. Spore yield was strongly influenced by weather conditions. In 1995, spots collected in the field, whose necrotic centre still produced conidiophores, were used. Afterwards, spots were treated in such a way as to remove conidia but preserve conidiophores or, at least, pseudostromata; sporulation was then stimulated in the laboratory. Under such conditions, the spore yield certainly suffered from the effects of weather conditions which affected the production of spore-bearing structures in the field. In 1996, spots without any spore-bearing structures were used, being produced by artificial infection and constantly maintained under conditions which prevented any sporulating activity (Canova, 1959c). Therefore, spore yield occurred under optimal environmental conditions.

The level of resistance of sugar beet cultivars affected spore yield significantly: the more resistant ‘Monodor’ and ‘Break’ yielded fewer conidia per unit of necrotic area than the susceptible ‘Cyrano’ and the less resistant ‘Bushel’, the former cultivars producing on average in the three experiments about 35% of the conidia yielded by the latter ones (Table 1). Differences between cultivars may be considered consistent over experiments for both their rating and significativity. In fact, cultivars rated the same only in a few cases, during surveys carried out in 1994 under field conditions, when meteorological conditions did not favour sporulation. Differences between cultivars were also consistent between the times when spots were induced to sporulate, so that even after 6 hours ‘Cyrano’ spots produced more conidia than the spots of the most resistant cultivars.

The effect of a reduced spore production on disease progress at the field level should be further investigated. In fact, it could strongly reduce the magnitude of resistance in the experimental fields where many cultivars, both susceptible and resistant, are grown close to each other, because of interplot interference (Parlevliet and Van Ommeren, 1975).

The morphology of the conidia was influenced by host resistance, so that conidia produced on the susceptible ‘Cyrano’ were longer, wider, and had more cells than conidia yielded on the resistant cultivars, irrespective of their resistance level. All the above-mentioned differences were small, though significant. Differences in the morphology of conidia borne on various host plants has been reported by Canova (1959a), but they were related to host species rather than to sugar beet cultivars.

Conidia produced on ‘Cyrano’ spots germinated more quickly than conidia yielded on other cultivars, especially ‘Monodor’ and ‘Break’, and germ tubes produced by germinating conidia grew more rapidly. We did not find any previous information about germination of C. beticola conidia depending on host resistance, but it has been demonstrated that the source of nutrient, for example the status of leaf conservation or the composition of an artificial medium, influenced both spore morphology (Canova, 1959a) and spore germination (Canova, 1959b). In addition, differences in spore germination have been attributed to differences in the age of conidia. Canova (1959b) showed that young conidia (6 to 15 hours-old) germinated more slowly than older ones (22 to 72 hours-old). The latter consid-
eration can help explain germination differences observed in the present work on various cultivars. In fact, populations of conidia obtained from different cultivars were not of the same age. When spots of the susceptible cultivar had been stimulated to sporulate they quickly produced more conidia than spots of a resistant cultivar; thus, at any one time during sporulation a great part of the conidia produced on spots of the former cultivar were older than conidia yielded on the latter cultivar.

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