CERCOSPORA LEAF SPOT: THE EFFECTS ON SUGAR CONTENT REDUCTION

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ABSTRACT

The damages caused by Cercospora leaf-spot to the sucrose production and to the sugar content have been studied with three-year trials (2001-2003) in three North Italian localities. Different disease severities were achieved by means of varieties having a different genetic tolerance to the disease and of three protection levels with fungicides. Production and quality data were appraised in three harvest periods. The integrated plant protection measures, as normally used, proved to have reduced by 76,4% the disease-induced total damages to the sugar content, and by 79,0% the total damages caused to raw sucrose. Considering the maximum protection, Cercospora proved to affect a sugar-content reduction of 18,2% in the susceptible variety, and of 18,0% in the tolerant variety.

CERCOSPORIOSE: EFFETS SUR LA REDUCTION DE LA RICHESSE

ABREGE

Le dommages causés par la cercosporiose à la production de saccharose ont été évalués par des essais triennaux, réalisés dans trois localités de l'Italie du Nord. Afin d'obtenir de situations d'intensité différentes de maladie, on a employé des variétés ayant une différente tolérance génétique à la cercosporiose, et avec trois niveaux de protection par fongicides. Les données productives et qualitatives ont été évaluées en trois époques de récolte. Les mesures de lutte intégrée, employées habituellement, ont réduit du 76,4% les dommages totaux causés par la maladie sur la richesse, ainsi que du 79,0% sur le saccharose brut. Si on considère la protection maximale, la cercosporiose s'est démontrée capable d'agir sur la réduction de la richesse dans l'ordre du 18,2% pour la variété sensible, et du 18,0% pour la variété tolérante.

CERCOSPORA: AUSWIRKUNGEN AUF DEN ZUCKERGEHALTRÜCKGANG

KURFASSUNG

Die Schäden, die von der Cercospora dem Saccharose-Ertrag und der Polarisation verursacht werden, wurden mit Versuchen von drei Jahren untersucht, die in drei norditalienischen Orten stattfanden. Um Umgebungen mit verschiedener Krankheitsintensität zu erzielen, wurden Varietäten mit unterschiedlicher, genetischer Toleranz gegen Cercospora verwendet sowie drei Schutzstufen mit Fungiziden. Die Produktions- und Qualitätsdaten wurden für drei Erntereifen ermittelt. Die normal eingesetzten integrierten Pflanzenschutzmaßnahmen haben um 76,4% die durch die Krankheit

entstandenen Gesamtschäden an der Polarisation reduziert, sowie um 79,0% die Gesamtschäden an der Saccharose. Angesichts des eingesetzten Maximalschutzes ist festzustellen, daß die Blattfleckenkrankheit (Cercospora) mit etwa 18,2% an der Reduzierung der Polarisation in der empfindlichen Varietät und mit 18,0% an der toleranten Varietät beteiligt ist.

INTRODUCTION

Cercospora leaf spot (CLS) is the worldwide most common and noxious fungal disease of the sugar beet (*Beta vulgaris L.*). It appears on the leaf blade with typical necrotic spots. In more severe cases, these spots expand and cause the beet leaves to dry up. This disease is propagated by the fungus *Cercospora beticola Sacc*. and is spread with variable intensities in all areas with a temperate climate, aside from the countries at extremes south or north of beet cultivation range. The damages to the production are reportedly increasing in several Central European countries that in the past had been affected only marginally (HOLTSCHULTE, B., 2000). In Italy, CLS finds favourable development conditions on nearby 80% of the areas cultivated with beets. However, the most harmful attacks are mainly signalled in the area north of the Po river (CIONI, F. *et al*., 1996; ROSSI, V. *et al*., 1995). Every year this infection grows in intensity, mainly in relation with the changeable climate conditions. However, many variables contribute to the final production damage: the sensitivity of variety used, the timing of first infection, the timeliness and effectiveness of the protection programs, the irrigation, the harvesting time, the simultaneous presence of abiotic stresses and other plant diseases etc. The control of this disease is based on these measures: use of resistant varieties, repeated spraying of fungicides, long rotations to reduce environmental *inoculum* in farmlands, early harvests to prevent more serious consequences of infections. With an integrated protection, under normal working conditions, it is possible to cancel approx. two-thirds of the potential damages (BIANCARDI, E., 1998). Yield loss depends primarily on the reduced photosynthetic function of the assimilating surface. A severe attack to the foliage will determine a major growing of new leaves: a reaction called 'regrowth' (SHANES, W.W. & TENG, P.S., 1992; STEVANATO, P. *et al.* 2001).

The sugar content reduction, i.e. the reduction of sugar content in the beet during its harvest is a typical problem of the Italian beet growers (MUNERATI, O., 1920). It presently represents a major challenge in the effort of raising sugar yields per hectare. In fact, if compared to a general gain in the period August-October, while in other Northern European countries also sugar content keeps increasing or remaining constant, in Italy a loss is usually registered, that counteracts the increase of the root weight. The loss of sugar content is mainly influenced by environmental and agronomic factors. As for the first ones, the climatic developments in Italy, featured by hot and dry, nearly unbroken summers and, end of August/beginning of September, by a sudden change, with an brusque temperature drop and heavy precipitations, produced scarcely favourable conditions for beet growth (CASARINI, B. *et al*., 1999). These relatively late stresses slow the growth of the leaves, causing their premature senescence, reduced interception of the light radiation and reduced conversion coefficient of radiation. Moreover, the sugar beet is a C3 plant. Therefore, in a hot dry environment its energetic expenditure (represented by the loss linked to photorespiration) is often very high, higher than that of the C4 plants. That is why we can have years being more or less favourable to sugar accumulation in the root and to the upkeep of sugar at high levels (PERATA, P., 2002). There are years, in which sugar content has less unfavourable progressions, and there are years significantly marked by sugar content reduction. In more favourable years, approx. 1,5 degrees of sugar content can be lost along the harvest season, while in the less favourable more than 2-3 degrees (CIONI, F., 2003).

As for the agronomic technique, the sugar-content level in the root should be considered as a result of the complex course where several factors interact. In particular, variety aspect, CLS and nitrogen fertilisation certainly play a major role, but their combined action is probably more effectual because they affect the plant by varying the growth dynamics of the leaf apparatus, thus significantly influencing the saccharose accumulation in the root (MERIGGI, P., *et al.*, 2003).

Purpose of this work: a) quantifying CLS influence on sugar-content reduction in two different levels of genetic resistance to the disease, and b) determining the effectiveness (with respect to the theoretical levels) of an integrated plant protection as recommended by the National Technical Commission of beet growers and sugar industries.

1.- MATERIAL AND METHODS

The trials took place in the years 2001, 2002 and 2003 in 3 localities: Bologna, Ferrara and Rovigo. These belong to 2 homogeneous areas distinguished for the different intensity of CLS: medium-high (Bologna) and high intensity (Ferrara and Rovigo). Trial layout included a comparison between 4 highly marketable varieties featured by similar productive characteristics, a different (scarce, null or medium) resistance to CLS and a good resistance to rhizomania. Different infection levels were obtained by utilising 3 protection programs with fungicides: PO = untreated; P1 = protection every 20 days beginning with the first appearance of the spots, according to the plan of the National Technical Commission (CANOVA A. *et al.* 1996); P2 = double protection, every 10 days, starting from same date and with the modalities of P1. Level P2 simulated a nearly absence of the disease in order to obtain the best possible quantification of the production losses caused by the pathogen. The treatments were implemented with difenoconazol + fenpropidine and trifloxystrobin + cyproconazol, both with 700 g/ha of a commercial formulation (Spyrale and Sphere) by spraying a water volume of 500 l/ha. At first appearance of the disease, the severity of CLS infections was periodically evaluated through a dedicated assessment scale, on a sample of plants (situated in the central part of each plot): a value ranging from 0 (healthy plant) to 5 (plant with necrotised leaf apparatus) was attributed. The severity values of CLS were then expressed as a percentage of the affected leaf area (A.L.A., in a scale from 0 to 100) through a regression equation (MERIGGI, P. *et al.*, 1998). Of the evaluations carried out at two-week intervals, those that had been realised at each harvest are considered in the present paper, since they are more correlated with the yield data.

In the single trials, varieties and chemical treatments were put in a split-plot trial design with 4 repetitions. The treatment plots were submitted to 3 fungicide treatments (P0, P1 and P2), the subplots to the varieties (S and R) and the sub-subplots to 3 harvesting times (H1, H2 and H3). The elementary plot was 25 m^2 wide, with a harvest surface of at least 6.3 m^2 , in order to have a sufficient number of roots for productive and technologic analyses (AMADUCCI, M.T. *et al.*, 1982). The samples were subjected to quantitative-qualitative laboratory analyses. The harvests took place on August 10-15, September 15-20, and September 25-October 5, i.e. they were representative for the entire harvest season. The data of the 3-year period were subjected to a variance analysis based on the adopted experimental design. These factors were considered: protection program (3), variety (4) and harvest time (3). For each parameter and before the cumulative elaboration, the homogeneity of the error variances in different years was verified. The average values were separated by applying the Student-Newman-Keuls test with P 0.05.

The evaluation of CLS impact on sugar yield and content was calculated considering P2 yield production as healthy plots. Thereafter maximum disease damage was obtained from difference between RP2 and SP0.

1.1.- RESULTS

In the 3 years of trial, the first spots of the disease appeared between June 14 and June 18 in Rovigo and Ferrara, and 5-8 days later in Bologna. Treatments started in the same periods. The epidemics had different evolutions as a result of the atmospheric condition during each trial (year and site): A.L.A. values in the untreated controls of the susceptible varieties ranged from a minimum of 41.9 (Bologna, 2002) to a maximum of 63.3 (Rovigo, 2003). The effects of the fungicides in slowing down the disease progression was evident (Table 1). As a whole, P1 had an efficiency of 75% in reducing P0 A.L.A. to a level of 6.7, while P2 of 89% to 2.7. This effectiveness was apparently affected by the interaction with the harvest time. In the first two harvests, P1 and P2 had a reciprocally similar effect, with differences of 0.2 A.L.A. (equal to an average effectiveness of 59% on P0). This difference increased, during the harvest season, to 10.9 (with an effectiveness of 70% for P1, and of 90% for P2 over P0).

Table 1. *Affected Leaf Area (%) of Cercospora leaf-spot epidemics measured for each field trial during the harvest season (variety mean values).*

Table 1. *Surface foliaire atteinte (%) par la cercosporiose, mesurée sur chaque parcelle d' essai pendant la campagne de récolte (moyennes variétales).*

Tafel 1. *Von der Cercospora befallene Blattfläche (%), gemessen je Versuchsfeld während der Ernte (Durchschnitt je Sorte).*

Harvest time	Spraying programs	Site and year					Trial means
		BO 01	FE 01	BO 02	FE 02	RO 03	
1st	P0	1.76	1.91	1.69	1.83	1.89	1.82
	P1	0.97	0.78	0.63	0.78	0.98	0.83
	P2	0.65	0.69	0.44	0.52	0.98	0.66
2nd	P0	25.88	29.62	15.31	18.65	31.92	24.28
	P1	3.88	2.43	1.98	1.54	4.68	2.90
	P2	2.13	2.01	1.67	1.21	2.98	2.00
3st	P0	56.59	59.11	41.89	48.41	63.31	53.86
	P1	15.04	17.47	10.20	18.34	20.70	16.35
	P2	5.22	7.38	3.09	6.22	5.29	5.44
Treatment means	P0	28.08	30.21	19.63	22.96	32.37	26.65
	P1	6.63	6.89	4.27	6.89	8.79	6.69
	P2	2.67	3.36	1.73	2.65	3.08	2.70

BO 01=Bologna 2001; FE 01=Ferrara 2001; BO 02=Bologna 2002; FE 02=Ferrara 2002; RO 03=Rovigo 2003.

Table 2 shows an overview of the analysis of variance, highlighting the effects of the sources of variation and of their interactions on sugar content and on sugar yield. Year and site factors turned out to be partially significant. The absence of significant differences on sugar yield between different sites confirms the homogeneity of the crop reactions to the factors of variation in the monitored localities (according to: SMITH, G.A. & MARTIN, S.S., 1978; SMITH, G.A., 1985). The responses of the quantitative and qualitative parameters to harvest times (H), to protection programs (P) and to varieties are all highly significant.

Host resistance determined an increase of the average sugar content by 0.44 %, while the fungicide treatments P1 and P2 (as compared to P0) determined an increment of 1.28 and 1.89 respectively. In the untreated checks of the susceptible variety, sugar content was constantly reduced during the harvest season. However, both the genetic resistance (to a more limited extent) and the fungicide treatments (to a major extent) have significantly limited this cutback.

Table 2a. *Outline of the analysis of variance.*

Table 2a. *Résumé de l'analyse de variance.*

Tafel 2a. *Zusammenfassung Varianz-Analyse*

Source of variation	Sugar content (%)	Raw sugar yield ($t*ha^{-1}$)
Year	*	**
Locality	*	ns
Harvest time (H)	**	**
Treatment programs (P)	**	**
Variety	**	**
Harvest time x Treatment programs	**	**
Harvest time x Variety	**	**
Treatm. programs x Variety	*	ns
Harvest time x Treat. programs x Variety	Ns	*

Table 2b. *Mean separation*

Table 2b. *Séparation entre les moyennes*

Tafel 2b. *Trennung der Mittelwerte*

Harvest time		
H1 10-15 august	15.87 a	9.58 c
H2 15-20 september	15.11 b	10.46 b
H3 25 september-5 october	13.74 c	10.99 a
Treatment programs		
P0	13.85 c	8.95 c
P1	15.13 b	10.53 b
P2	15.74 a	11.54 a
Variety		
Susceptible	14.69 b	10.14 b
Resistant	15.13 a	10.54 a

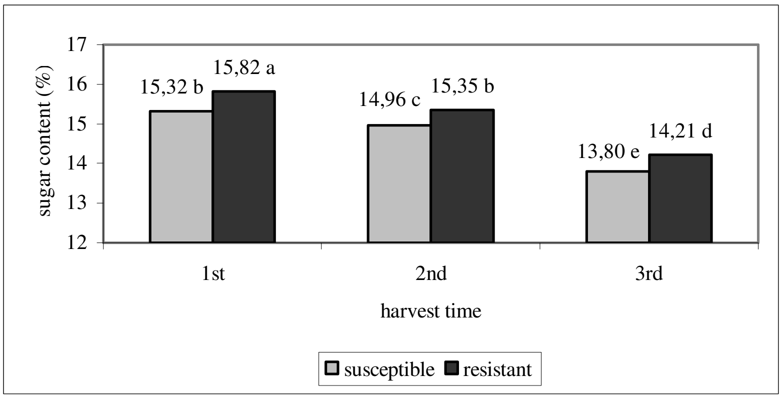
* $P \leq 0.01$; ** $P \leq 0.05$; ns not significant; means followed by the same letter are not significantly different at $P \leq 0.05$ (Student-Newman-Keuls test).

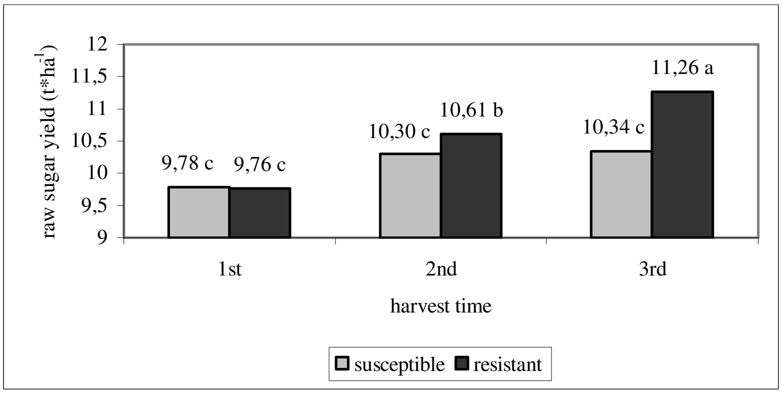
In fact, the monitored differences between resistant and susceptible variety, and between treated and untreated plots, showed increments during the harvest season (Fig. 1 and 2, above). Actually, the sugar content in the tolerant variety, for each of the 3 harvests was higher (as compared to the susceptible variety) by 0.50%, by 0.39% and of 0.41% (Fig. 1, above). Instead, P1 determined increments of respectively 0.73%, of 1.02% and of 2.09% (Fig. 2, above chart).

Figure 1. *Interactions between harvest time and genetic resistance to Cercospora leaf-spot in determining sugar content (above) and sugar yield (below). Figures followed by different letters are significantly different at P ≤ 0.05 (Student-Newman-Keuls test).*

Figure 1. *Interaction entre la période de récolte et la variété avec la cercosporiose dans la détermination de la richesse (en haut) et du rendement sucre (dessous). Les figures suivies par des lettres différentes sont significativement différentes en P ≤ 0.05 (Student-Newman-Keuls test).*

Fig. 1. *Wechselbeziehung zwischen Erntezeit und Sorte mit der Cercospora zur Ermittlung des Gehalts (ob.) und des Rohzuckerertrags (un.). Die Abbildungen, die von verschiedenen Buchstaben gefolgt sind, sind signifikant verschieden in P ≤ 0.05 (Student-Newman-Keuls test).*



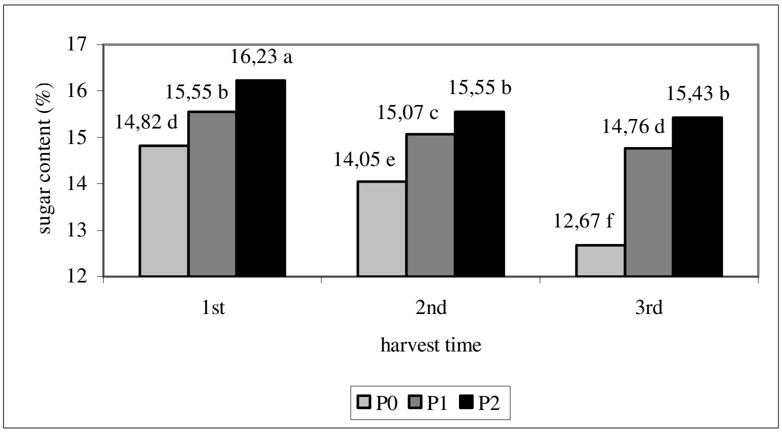


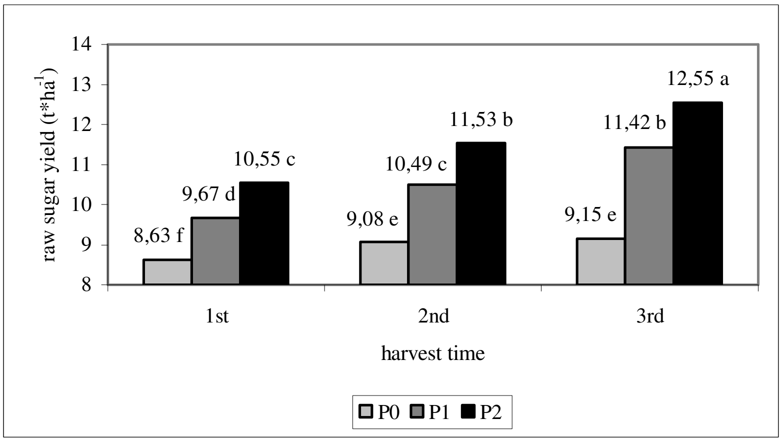
As a result of above effects, higher sugar yields (+0.40 t*ha^{-1}) were reported for the tolerant variety, as compared to the susceptible one. About chemical-treatment programs, P1 raised sugar yield by 1.58 t*ha^{-1}, and P2 by 2.59 t*ha^{-1}, as compared to the untreated crop, in agreement with other studies (ROSSI, V. *et al.*, 2000). In both cases sugar yield progressively increased during the harvest season, while in the susceptible variety it did not significantly rise after the end of August and in the untreated check after mid-September (Fig. 1 + 2, below chart).

Figure 2. *Interaction between harvest time and programs of treatments against Cercospora leaf-spot on sugar content (above) and raw sugar yield (below). Figures followed by different letters are significantly different at P ≤ 0.05 (Student-Newman-Keuls test).*

Figure 2. *Interaction entre la période de récolte et le programmes de traitements envers la cercosporiose sur la polarisation (en haut) et le rendement de sucre brut (dessous). Les figures suivies par des lettres différentes sont significativement différentes à P ≤ 0.05 (Student-Newman-Keuls test).*

Fig. 2. *Wechselbeziehung zwischen Erntezeit und Behandlungsprogrammen zur Cercospora-Bekämpfung bei Ermitteln von Zuckergehalt (oben) und Rohzuckerertrag (unten). Die Abbildungen, die von verschiedenen Buchstaben gefolgt sind, sind signifikant verschieden in P ≤ 0.05 (Student-Newman-Keuls test).*





In order to evaluate the different importance of both genetic tolerance and chemical protection programs, it has been possible to separate single effects on the two reference parameters: raw sugar yield and sugar content. Genetic tolerance raised raw sugar yield in the 3 programs by 0.67, 0.29 and 0.24 $t^{*}ha^{-1}$, respectively for P0, P1, and P2, while sugar content by 0.60, 0.44 and 0.28 %. Instead, the fungicide-based treatments influenced the raw sugar in the susceptible variety by 1.27 t*ha^{-1} in P1 and by 0.41 in P2, while they influenced the tolerant variety by 0.89 t*ha^{-1} in P1 and by 0.36 in P2. As for sugar content, they raised it by 0.88 % in P1 and by 0.58 % in P2 for the susceptible variety; by 0.72 in P1 and by 0.42 in P2 in the tolerant one (Fig. 3). Whenever both control measurements were applied simultaneously, their effect was additive. In fact, the P1-treated tolerant variety delivered a yield increase over the P0 susceptible one of 1,56 t*ha^{-1}, and an increase of sugar content of 1.32%, whereas for P2 the increments were 1.92 t*ha^{-1} and 1.74% respectively. This value is approximately equal to the arithmetic sum of the effects of both factors, if taken individually.

Figure 3. *Separate and combined effects of variety resistance and fungicide treatments against Cercospora leaf-spot on raw sugar yield (t*ha^{-1}) and on sugar content (%) of the sugar beet crop.*

Figure 3. *Effets séparés et combinés de résistance variétale et traitements contre la cercosporiose sur le rendement en sucre brut (t·ha^{-1}) et sur la richesse (%) de la betterave.*

Fig. 3. *Getrennte und kombinierte Wirkungen der Sortenresistenz et Fungizid-Behandlungen gegen Cercospora auf den Rohzuckerertrag (t·ha^{-1}) et Zuckergehalt (%) je Rübe.*

Raw sugar yield (t*ha^{-1})

Rendement de sucre brut (t*ha^{-1})

Rohzuckerertrag (t*ha^{-1})

	P0	***P1-P0***	**P1**	***P2-P1***	**P2**
Susceptible var. (S)	9.16d	*+1.27*	10.43b	*+0.41*	10.84a
R – S	*+0.67*		*+0.29*		*+0.24*
Resistant var. (R)	9.83c	*+0.89*	10.72a	*+0.36*	11.08a

Sugar content (%)

Richesse (%)

Zuckergehalt (%)

	P0	P1-P0	P1	P2-P1	P2
Susceptible var. (S)	13.91e	*+0.88*	14.79c	*+0.58*	15.37a
R – S	*+0.60*		*+0.44*		*+0.28*
Resistant var. (R)	14.51d	*+0.72*	15.23b	*+0.42*	15.65a

Means followed by the same letter are not significantly different at $P \leq 0.05$ (Student-Newman-Keuls test).

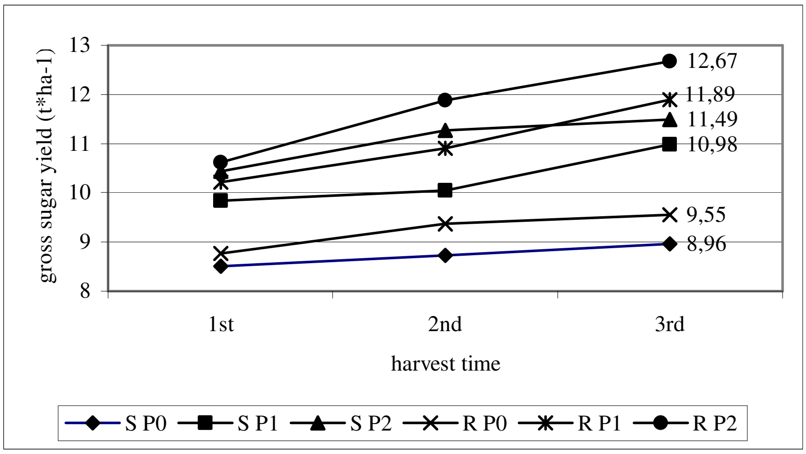
Fig. 4 shows the evolution of sugar yield and content as a function of chemical treatments and of genetic tolerance. If uncontrolled, either with treatments or with the tolerance, this disease can affect an appreciable reduction of sugar yield even at an early harvest (above chart). With program P1, the damage by the Cercospora (calculated as the difference between RP2 and SP0) in the susceptible variety is reduced by 54.5% at the date of the 3rd harvest. Whenever tolerant varieties are used, same value reaches 79.0 on the saccharose, and 76.4 on the sugar content.

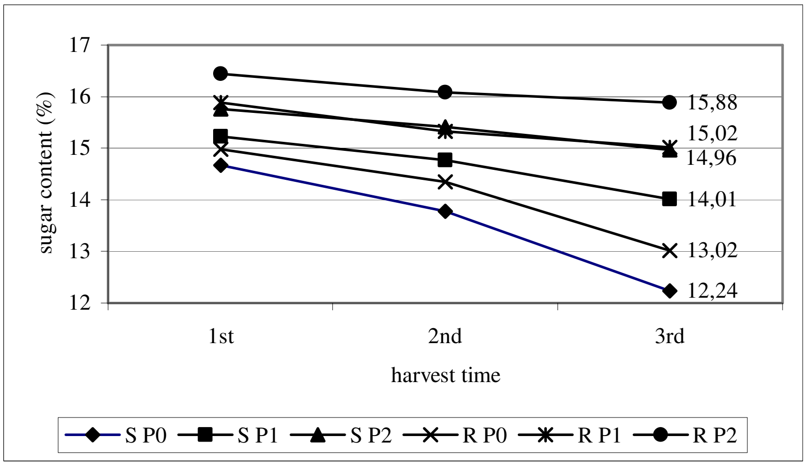
As for sugar-content reduction (Fig. 4, below chart), CLS had an influence at the date of the 3rd harvest time, namely with 18.2% (equal to 2,72 °S) in the susceptible variety, and with 18.0% (equal to 2,86 °S) in the tolerant variety. As a whole, comparing RP2 with SP0, the disease influenced with 22.9% (equal to 3.6 °S).

Figure 4. *Dynamics of sugar yield (t*ha^{-1}) (above) and sugar content (%) (below) in varieties (S=sensitive; R=resistant) under P0, P1 and P2 treatments against Cercospora leaf-spot. The differences are significant (for P ≤ 0.05, Student-Newman-Keuls test) starting from the first harvest time.*

Figure 4. *Dynamique de rendement sucre (t-ha^{-1} (en haut) et de richesse (%) (dessous) dans les variétés (S = sensible, R = résistante) dans les traitements P0, P1 et P2 contre la Cercospora. Les différences sont significatives (pour P 0.05, Student-Newman-Keuls) dès la première période de récolte.*

Fig. 4. *Entwicklung des Rohzuckerertrags (t-ha^{-1}) (oben) und Zuckergehalts (%) (unten) der Sorten (S = empfindlich, R = empfindlich) bei Behandlungen P0, P1 et P2 gegen Cercospora. Unterschiede sind signifikant (für P 0.05, Student-Newman-Keuls) beginnend von der ersten Erntezeit.*





CONCLUSION

The research gave the following results:

- Fungicide treatments proved to have an average reduction of A.L.A. (calculated on P0) of 74.9% with program P1 (CTN recommendations), and of 89.9% with P2 (maximum protection).

- On the whole, (comparing RP2 with SP0) the disease caused a sugar-content reduction of 3.6°S (22.9%). In detail, variety resistance determined an increment in sugar content of 0.44°S with respect to susceptibility, whereas the protection programs P1 and P2 incremented it by 1.28 and 1.89 respectively, with respect to P0.

- The recommended measures of integrated plant protection reduced the total loss of sugar content linked to the disease (calculated as the difference between RP2 and SP0) by 76.4%, and the loss of sugar yield by 79.0%. The residual damage does not always justify the difference in costs between protection programs P1 and P2.

- The residual damage does not always justify the difference in costs between protection programs P1 and P2.

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