LEAF DYNAMICS IN SUGAR BEET UNDER CERCOSPORA LEAF SPOT ATTACK

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The disease caused by the fungus *Cercospora beticola* Sacc. is widespread throughout many countries with temperate climates (Holtschulte, 2000). In order to reduce damage caused to sugar beet (*Beta vulgaris* L. ssp. *vulgaris*), resistant varieties and fungicides are used. In normal conditions, it is possible to prevent about two thirds of the potential sugar yield losses (Biancardi, 1998). The damages are due firstly to the reduction of leaf surface and later to the faster emission of new leaves (Shane and Teng, 1992; Biancardi *et al.*, 1999). This so-called "regrowth" takes place at the expense of dry matter accumulated in the roots. In the more serious cases, the loss of production appears to exceed the sum of the actions of the aforementioned factors. In fact, there might exist a negative effect caused by toxins originated from the infection processes (Rossi *et al.*, 1990).

The dynamics of sugar beet leaves have been studied in environments not diseased by cercospora leaf spot (CLS). In such condition, the effects of climatic and agronomic factors have been pointed out (Clark and Loomis, 1977; Theurer, 1979; Barbieri, 1983 a; Milford and Riley, 1980; Milford *et al.*, 1985 a,b,c,d). This study intends to clarify the effect of CLS on the leaf dynamics at different levels of integrated protection.

Materials and methods

The data were obtained from trials cultivated near Rovigo (Po Valley-Italy) in the years 1987, 1999 and 2000, on alluvial calcareous soil with high clay content. In the region CLS is endemic. Local growing techniques adapted to experimental trials were used to carry out the tests. In order to produce uniform infection, the trials were artificially inoculated with solution prepared and sprayed as described by Ruppel and Gaskill, 1970.

The 1987 tests provided an evaluation of the effects of fungicide treatments on leaf area, emission and duration. Observations were made on untreated (T0) and treated (T1) plots. The last were protected as in the normal practice, i.e. every 20 days after the first appearance of the spots on the leaves. In the test variety Novagemo was used, sown in randomised blocks with 4 replications. Beginning in early May, samples were harvested weekly in each replication and treatment (T0 and T1). On such date, 24 beets with average development were collected from the 8 plots containing about 63 plants each. After the elimination of the 9 largest and 9 smallest roots, 6 representative beets were selected. This system is necessary in order to reduce the variation between the plants in the plot, and especially between the observation periods. The area of each pair of leaves was determined using an area-meter LI-COR 3000 (Borrelli *et al.*, 1990).

The leaf dynamics were recorded weekly on 8 labelled plants per treatment (T0 and T1). These beets were chosen for the leaf development representative of the population. As soon as a new leaf was emitted it was numbered in order of its appearance. The leaves were considered dead when at least half of the blade was yellowed or dry (Milford *et al.*, 1985 b; Lee and Schmehl, 1988). The first spots caused by the fungus appeared on 24 June on the oldest leaves. On the same date, 1.2 kg/ha of Lostal fungicide was sprayed in the T1 plots. The treatment was repeated another 3 times, i.e. every about 20 days following the same procedure.
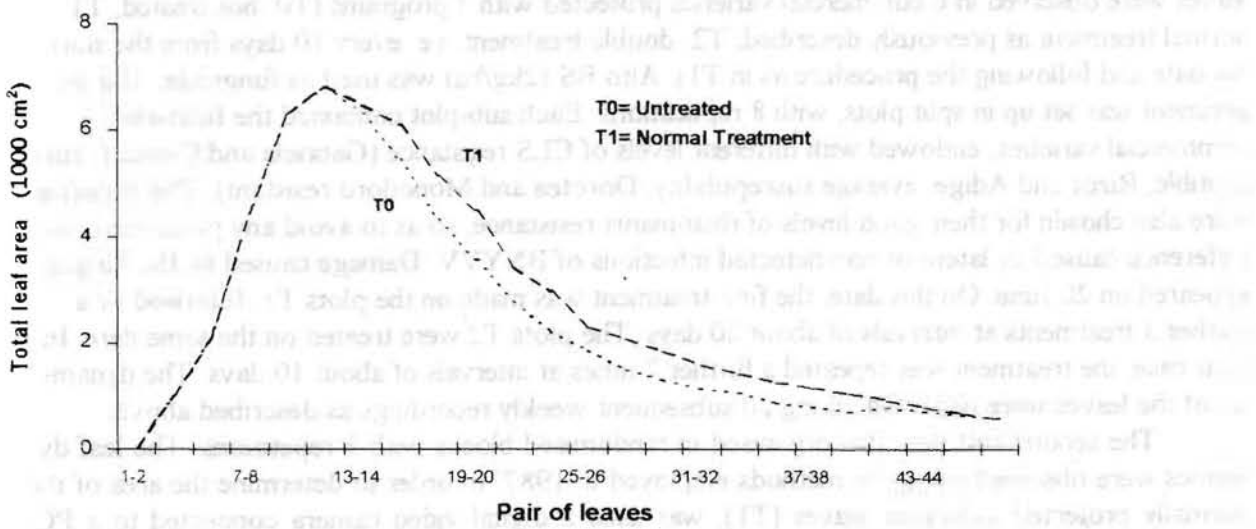
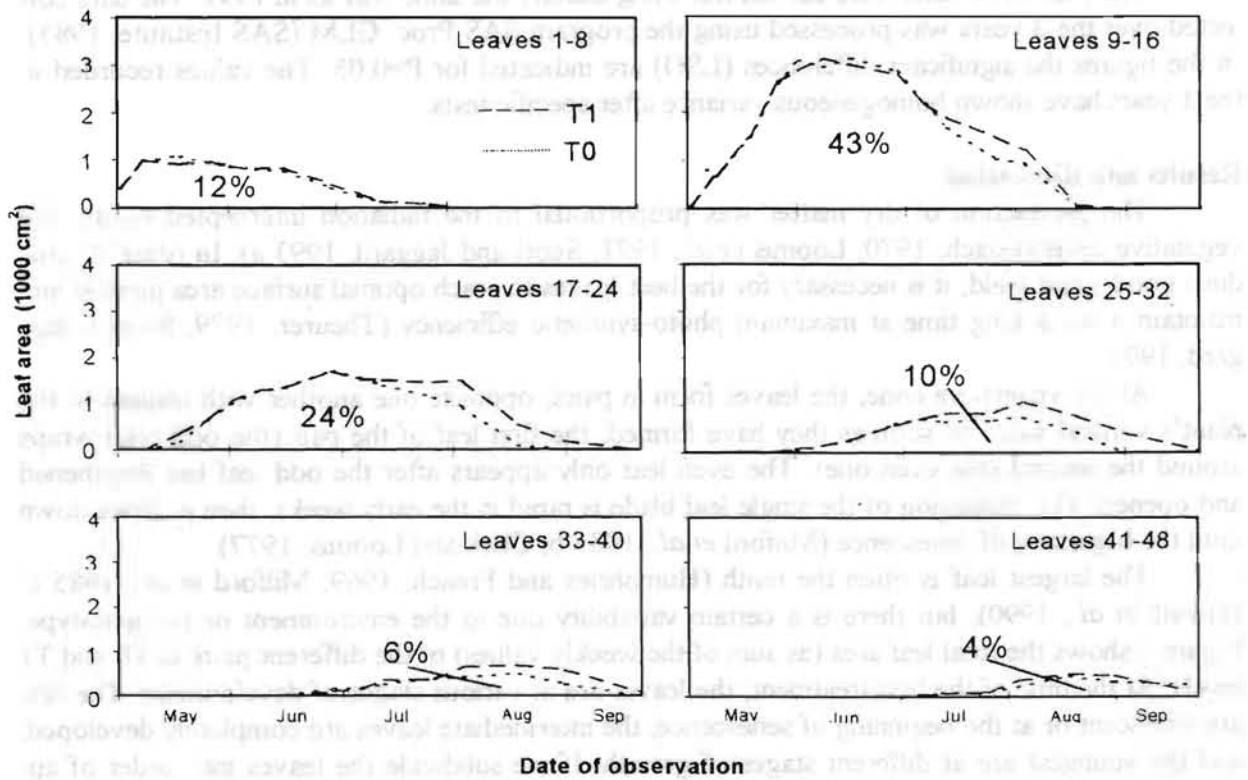Fig. 1: Changes in total leaf area per pair of leaves under the protection programs T0 and T1 (see text).

Fig. 2: Evolution of leaf area per groups of 8 leaves under the protection programs T0 and T1.

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In 1999, the tests were carried out in two separate fields. In the first, the dynamics of the leaves were observed in 6 commercial varieties protected with 3 programs (T0: not treated; T1: normal treatment as previously described; T2: double treatment, i.e. every 10 days from the starting date and following the procedure as in T1). Alto BS (2kg/ha) was used as fungicide. The experiment was set up in split plots, with 8 replications. Each sub-plot contained the following commercial varieties, endowed with different levels of CLS resistance (Gabriela and Contact: susceptible; Rizor and Adige: average susceptibility; Dorotea and Monodoro resistant). The varieties were also chosen for their good levels of rhizomania resistance, so as to avoid any productive interference caused by latent or non detected infections of BNYVV. Damage caused by the fungus appeared on 20 June. On this date, the first treatment was made on the plots T1, followed by a further 3 treatments at intervals of about 20 days. The plots T2 were treated on the same date. In their case, the treatment was repeated a further 7 times at intervals of about 10 days. The dynamics of the leaves were observed taking 20 subsequent weekly recordings as described above.

The second test was also organised in randomised blocks with 3 repetitions. The leaf dynamics were observed using the methods employed in 1987. In order to determine the area of the normally protected individual leaves (T1), was used a digital video camera connected to a PC, which could analyse the images using software developed by TECNA, Melara (Rovigo). The areas were converted into LAI (Leaf Area Index). For the density of 10 plants/m^2 as in the tests, this corresponds to the leaf area of a single beet expressed as 1000 cm^2 (Campbell and Viets, 1967). Leaf emission was expressed as LAR (Leaf Appearance Rate). In the definition given by Clark and Loomis (1977), the index represents the number of new leaves produced in a week.

The year 2000 tests were carried out using exactly the same way as in 1999. The data collected over the 3 years was processed using the program SAS Proc. GLM (SAS Institute, 1985). In the figures the significant differences (LSD) are indicated for P=0.05. The values recorded in the 3 years have shown homogeneous variance after specific tests.

Results and discussion

The production of dry matter was proportional to the radiation intercepted during the vegetative cycle (Loach, 1970; Loomis et al., 1971; Scott and Jaggard, 1993 a). In order to produce good sugar yield, it is necessary for the beet leaves to reach optimal surface area quickly and maintain it for a long time at maximum photo-synthetic efficiency (Theurer, 1979; Scott e Jaggard, 1993 a).

At the vegetative cone, the leaves form in pairs, opposite one another with respect to the plant's vertical axis. As soon as they have formed, the first leaf of the pair (the odd one) wraps around the second (the even one). The even leaf only appears after the odd leaf has lengthened and opened. The expansion of the single leaf blade is rapid in the early weeks, then it slows down until the beginning of senescence (Milford et al., 1985 b; Clark and Loomis, 1977).

The largest leaf is often the tenth (Humphries and French, 1969; Milford et al., 1985 c; Borrelli et al., 1990), but there is a certain variability due to the environment or the genotype. Figure 1 shows the total leaf area (as sum of the weekly values) of the different pairs at T0 and T1 levels. At the time of the first treatment, the leaves are at various stages of development. The first are senescent or at the beginning of senescence, the intermediate leaves are completely developed, and the youngest are at different stages of growth. If we subdivide the leaves into order of appearance and into groups of arbitrary consistency, as suggested by Humphries and French (1965), is possible to identify their contribution to the area produced in the whole cycle (Fig. 2). The second group of 8 leaves alone, i.e. from the 9th to 16th, forms 43% of the total area.

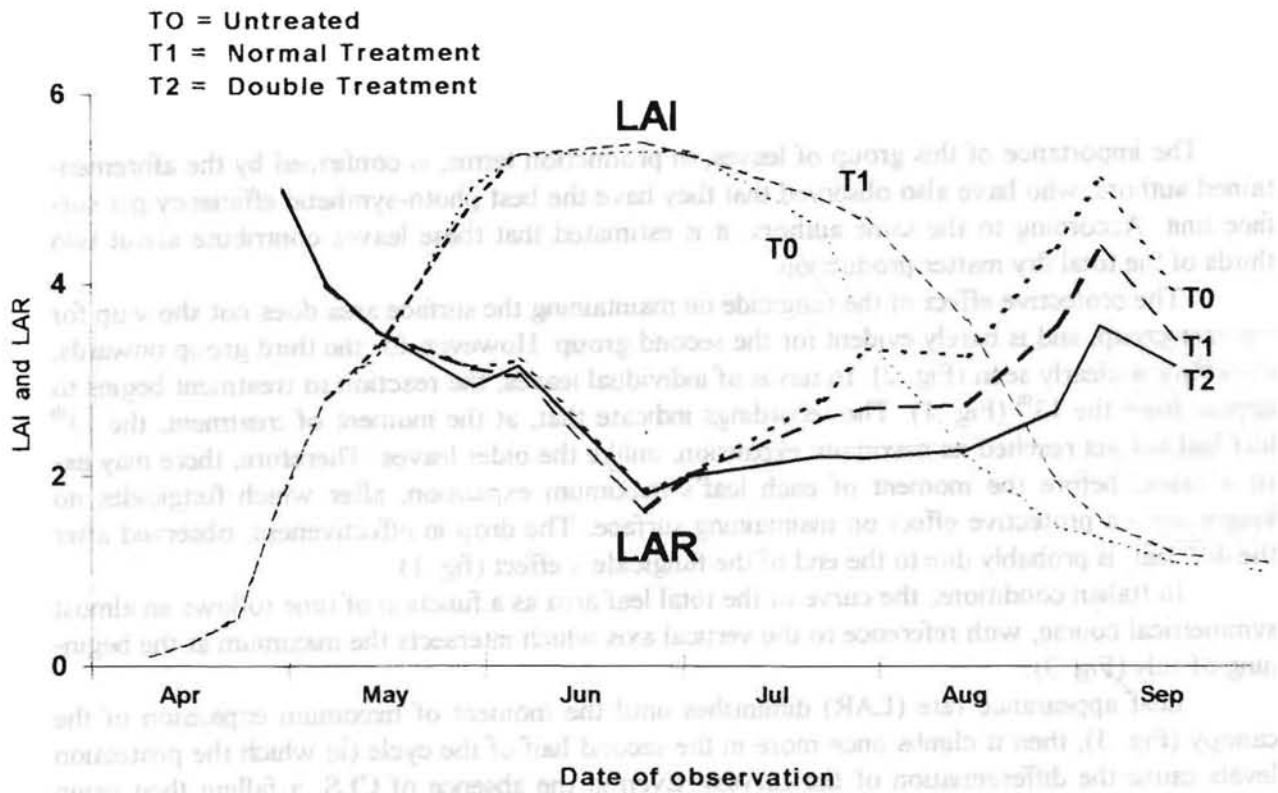Fig. 3: Evolution of LAI and LAR with different protection programs T0, T1 and T2 (see text). The LAI differences are significant (P= 0.05) from 9 August; the LAR differences are significant (P= 0.05) on 10 September.
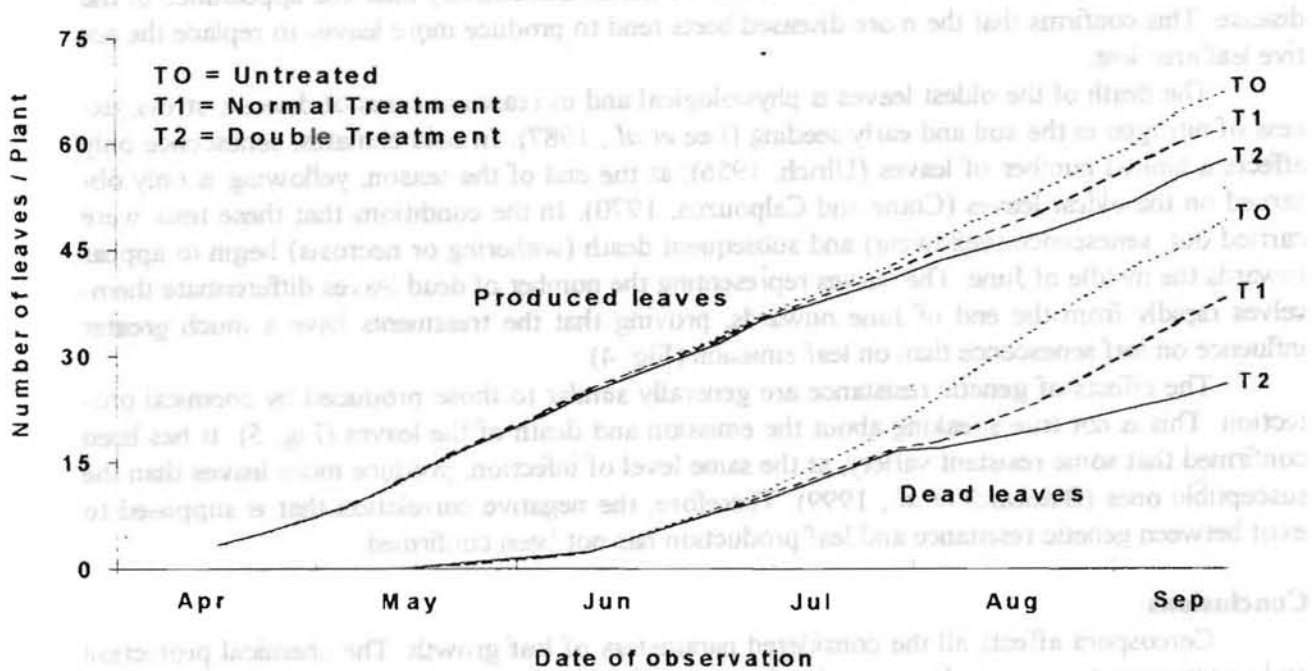

Fig. 4: Rate of leaf production and survival of leaves of 6 varieties under 3 protection programs (T0, T1, T2); the differences are significant (P= 0.05) beginning from 8 August for dead leaves and from 23 August for produced leaves.

The importance of this group of leaves, in production terms, is confirmed by the aforementioned authors, who have also observed that they have the best photo-synthetic efficiency per surface unit. According to the same authors, it is estimated that these leaves contribute about two thirds of the total dry matter production.

The protective effect of the fungicide on maintaining the surface area does not show up for the first group, and is barely evident for the second group. However, for the third group onwards, the effect is clearly seen (Fig. 2). In terms of individual leaves, the reaction to treatment begins to appear from the 13th (Fig. 1). The recordings indicate that, at the moment of treatment, the 13th leaf had not yet reached its maximum expansion, unlike the older leaves. Therefore, there may exist a stage, before the moment of each leaf's maximum expansion, after which fungicides no longer have a protective effect on maintaining surface. The drop in effectiveness, observed after the 40th leaf, is probably due to the end of the fungicide's effect (fig. 1).

In Italian conditions, the curve of the total leaf area as a function of time follows an almost symmetrical course, with reference to the vertical axis which intersects the maximum at the beginning of July (Fig. 3).

Leaf appearance rate (LAR) diminishes until the moment of maximum expansion of the canopy (Fig. 3), then it climbs once more in the second half of the cycle (in which the protection levels cause the differentiation of the curves). Even in the absence of CLS, a falling then rising curve has been observed, in other countries, as indeed has a curve with a second fall towards the end of September (Clark and Loomis, 1977). The LAR of the treatment T0 recorded on 16 September is more than one leaf per week greater than for the T1. This is probably a sign of regrowth.

Figure 4 shows how many leaves were produced and how many died on the different dates in the 3 levels of protection. The first curve differentiates immediately after the appearance of the disease. This confirms that the more diseased beets tend to produce more leaves to replace the active leaf area lost.

The death of the oldest leaves is physiological and increases in cases of disease, stress, excess of nitrogen in the soil and early seeding (Lee et al., 1987). In cold climates, senescence only affects a limited number of leaves (Ulrich, 1956); at the end of the season, yellowing is only observed on the oldest leaves (Crane and Calpouzos, 1970). In the conditions that these tests were carried out, senescence (yellowing) and subsequent death (withering or necrosis) begin to appear towards the middle of June. The curves representing the number of dead leaves differentiate themselves rapidly from the end of June onwards, proving that the treatments have a much greater influence on leaf senescence than on leaf emission (Fig. 4).

The effects of genetic resistance are generally similar to those produced by chemical protection. This is not true speaking about the emission and death of the leaves (Fig. 5). It has been confirmed that some resistant variety, at the same level of infection, produce more leaves than the susceptible ones (Biancardi et al., 1999). Therefore, the negative correlation that is supposed to exist between genetic resistance and leaf production has not been confirmed.

Conclusions

Cercospora affects all the considered parameters of leaf growth. The chemical protection and genetic resistance compliment each other as they are both necessary to maintain almost normal levels of photo-synthetic activity. The treatments limit the loss of active surface area, reduce the Leaf Appearance Rate, increase the life span of the leaves and noticeably delay the senescence processes. The genetic resistance and the normal chemical defence have similar effects. However, the former is generally less effective.
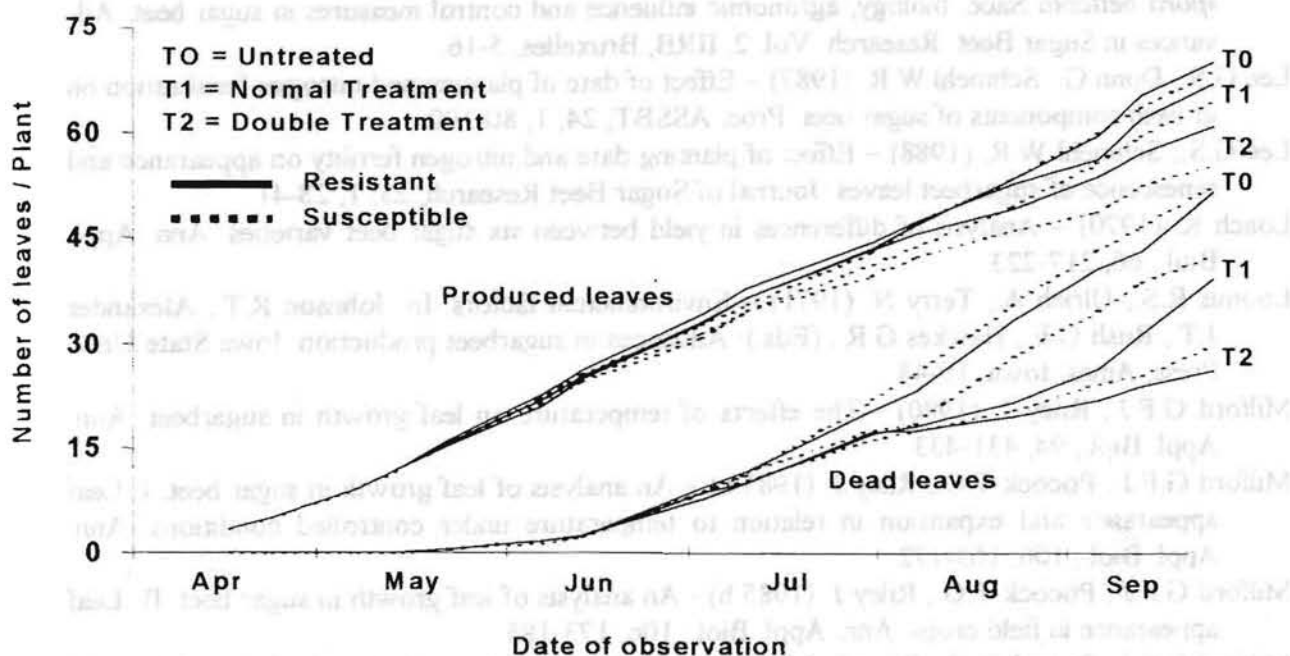
Fig. 5: Rate of leaf production and survival of leaves of susceptible and resistant varieties under 3 protection programs (T0, T1, T2).

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