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Leaf Tissue Senescence

CONSTANT RESPONSIVENESS TO HORMONES DESPITE A SEASONAL CYCLE IN SENESCENCE RATE[1]

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ABSTRACT

During winter, excised leaf tissue from *Rumex obtusifolius* degrades chlorophyll at twice the summer rate but the plant hormones, gibberellic acid and zeatin, inhibit the senescence rate by a constant percentage, regardless of season.

Three chemically unrelated classes of plant growth regulators are under investigation as physiological inhibitors of the senescence of higher plant tissues. In a wide variety of plants, the application of a cytokinin, a gibberellin, or an auxin retards the senescence or yellowing of leaf tissue.

It has been claimed that the responsiveness of leaf tissue to applications of these compounds varies with season, with a large retardation of senescence notable in the winter and a smaller one in the summer (1, 3, 8). An auxin was found to effect a retardation of senescence more quickly in older leaves of *Prunus* sampled in autumn than in young leaves sampled in spring and summer (8); it has been stated that gibberellic acid retards leaf senescence in *Taraxacum* at all seasons but that the effect is maximal in winter and early spring (3); the effect of GA_3 and kinetin on leaf tissue senescence of *Tropaeolum* was reported to be twice as great when plants were grown during short days than when grown during long days (1). This last and most quantitative report used as its effectiveness parameter the ratio of the amount of Chl remaining after five days in hormone-treated tissue to that in water-treated, control tissue.

We report in this paper that these apparent seasonal variations in leaf tissue responsiveness in *Rumex* are artifacts of the method used and disappear if the response is measured as a proportion of the senescence rate of control tissue. We here present what we believe to be the first quantitative description of a seasonal senescence cycle in excised mature leaf tissue.

MATERIALS AND METHODS

As we reported earlier (6), the reciprocal of the time to 50% Chl breakdown ($1/T_{50}$) can be a good measure of the rate of the over-all senescence process in leaf tissue from *Rumex obtusifolius* L. It is low in variability and has contributions made to it by both the slow phase of Chl breakdown and by the subsequent rapid or logarithmic phase of breakdown. This composite rate ($1/T_{50}$) is determined by floating surface-sterilized leaf discs of 6-mm diameter from mature fully expanded leaves on 1 or 2 ml of filter-sterilized hormone solutions in sterile plastic Petri dishes, and measuring the time course of Chl breakdown. Six to eight dishes of 10 discs each were incubated in darkness at 30 C. Two dishes were sampled at each time point during the rapid phase of Chl degradation and Chl was extracted and measured (4). We used a computer program to calculate the least square linear regression line of Chl breakdown, the composite rate, the first order rate constant (k), and the standard error of these last two rate measurements. Mosteller and Tukey's jackknife statistical method was used to calculate the standard error of the composite rate and of the first order rate constant (7). A complete description of the methodology is found in our previous paper (6). The data in part A of Figure 3 were taken from Chl breakdown lines.

Plants were grown under natural daylength at a temperature of 22 to 30 C in a greenhouse. Under these conditions of changing photoperiod the plants exhibited a seasonal variation in senescence of excised mature leaf tissue and in the rate of production of new leaves.

RESULTS

Figure 1 demonstrates a clear seasonal cycle in the rate of Chl breakdown of control leaf tissue incubated on water. During August, tissue senesces at less than half the winter rate. During the cycle observed, the minimum and maximum rates occurred at approximately the time of maximum and minimum yearly daylengths, respectively (Jun. 21, Dec. 21).

Figure 2 shows the time course of Chl breakdown for samples of mature leaf tissue taken in February and August and incubated on water or gibberellic acid. As previously reported (5), an initial slow phase was observed during which 10 to 20% of the Chl was degraded, followed by a rapid logarithmic phase of breakdown. For purposes of illustration, dashed lines are used to extrapolate the solid log-phase breakdown lines back to the slow phase. The slow phase of Chl breakdown was drawn to extend from 100% to 85% of initial Chl.

As shown in Figure 2, if samples of tissue were taken in February and allowed to senesce for 3 days, for example, there was twice as much Chl in GA_3-treated tissue than in tissue incubated on water (70% and 35% of initial, respectively). In August, however, the amounts were about equal because both hormone-treated and control tissue were still in the slow phase of breakdown. (If the incubation continued,

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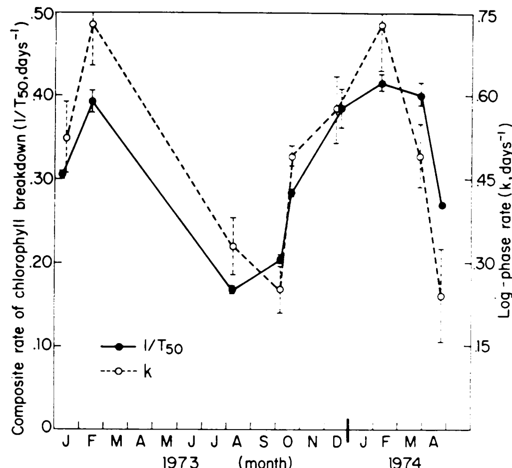

FIG. 1. Seasonal cycle in senescence rate of excised leaf tissue of *Rumex*. Leaf discs were incubated on H_2O in darkness at 30 C. Vertical bars represent two standard errors.

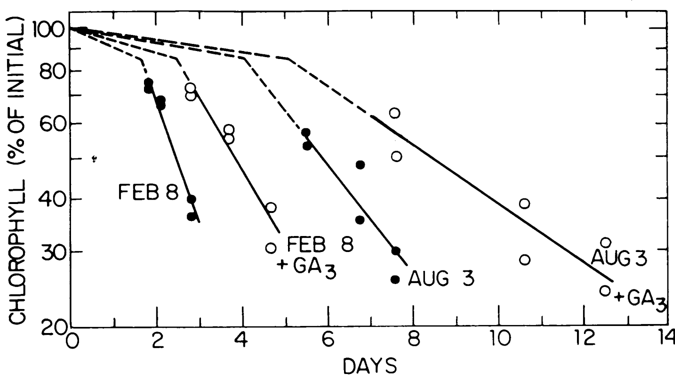

FIG. 2. Time course of Chl breakdown in *Rumex* leaf tissue in February and August. Leaf tissue was incubated on water (●), or 0.001 μM gibberellic acid (○).

however, a strong gibberellin effect was observed). Such comparisons of tissue Chl remaining after a fixed incubation period have often been expressed as percentage of Chl remaining in the control (Fig. 3A, discussed below).

Data obtained for gibberellin and cytokinin effectiveness at 3 times of the year are graphed in Figure 3. In graph A1, the dose response curves for GA_3 are distinct, and one might erroneously conclude that the hormone is much more effective in February than in August. A similar phenomenon was observed with zeatin treatment (A2). These measurements, however, are not rate measurements.

Alternatively, parts B1 and B2 of Figure 3 present data for tissue senescence rates from these same experiments. At all seasons tested and for both hormone classes, these rates decreased as hormone concentration increased.

Finally, to eliminate the variable of inherent seasonal differences in senescence rate observed in the water treated-leaf discs, the Chl breakdown rates are expressed as a percentage of the control rate (parts C1 and C2). The curves then become insignificantly different. The *P* value, found by means of the Student's *t* test, for the difference between the rates at 0.001 μM GA_3 for February and August is 0.8. The *P* value for the difference of the rates at 0.1 μM zeatin on October 6 and October 19 is 0.6 and for the difference of the rates at 10 μM zeatin on October 6 and March 26 the *P* value is 0.4.

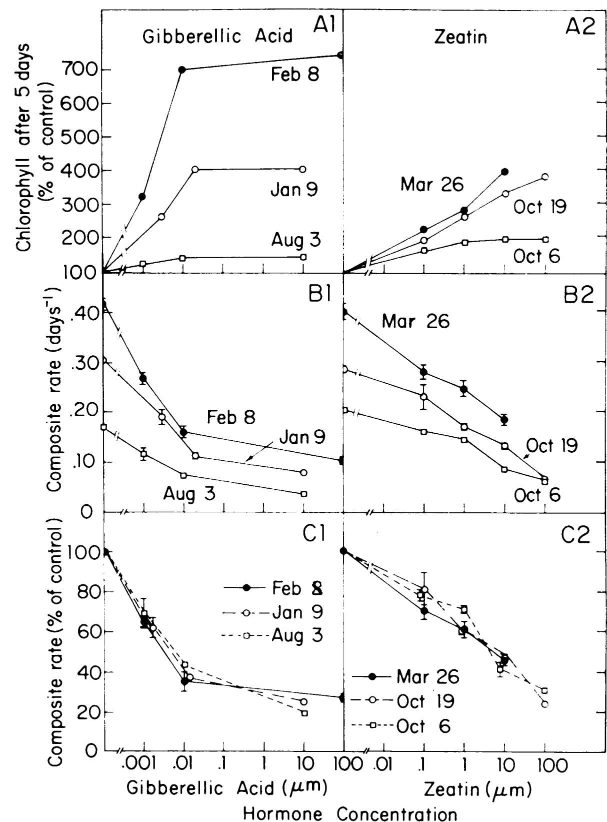

FIG. 3. Three ways of expressing hormone effectiveness in the retardation of senescence as a function of season. A: Chl level is measured after 5 days of incubation and plotted as percentage of control. B: Non-normalized rates of Chl breakdown are plotted. C: Rates of Chl breakdown are plotted as percentage of water control rate. Vertical bars represent two standard errors; where no bars appear, the standard errors are smaller than the symbols. No standard errors were calculated for part A.

DISCUSSION

We conclude that although there is a clearly demonstrable seasonal cycle in the rate at which excised leaf tissue ages on water, there is no change in hormone sensitivity with season. This implies that the amplitude of the hormone response mechanism which leads to an inhibition of senescence does not change.

The basis for the intrinsic fluctuation in tissue senescence rate with season might be caused by hormonal or other factors. Thus, leaf tissue grown under the long days of summer could contain higher amounts of senescence-inhibiting gibberellins (2), zeatin (9), or carbohydrate; or lower amounts of the senescence accelerator abscisic acid (10). The second of these alternatives seems unlikely in view of the demonstrated rapid inactivation of zeatin by *Rumex* leaf tissue (6).

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