the formation of new tubers on the sprouts, which demonstrate the incubation state reached.

Results and discussion. The period of incubation of tubers formed on both temperatures are shown in the Table.

Tubers formed at 27 °C were classified into 2 groups: normal and deformed tubers with secondary growth. Observations were made periodically, maintaining the vermiculite humid and checking the formation of little tubers on the sprouts. The date of tuber formation was taken when on the buds of the sprouts or on rhizomes little tubers of 3 mm diameter appeared (Table).

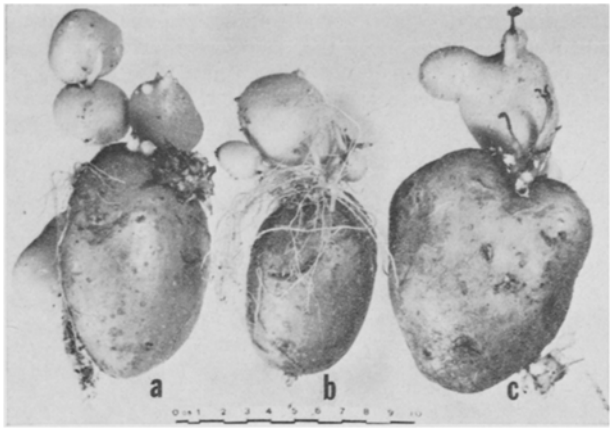

Fig. 2. Tubers formed at 27 °C after 33 days of initiation of tuberization. a) Secondary tuber formation; b) normal tuber; c) tuber formed showing second growth.

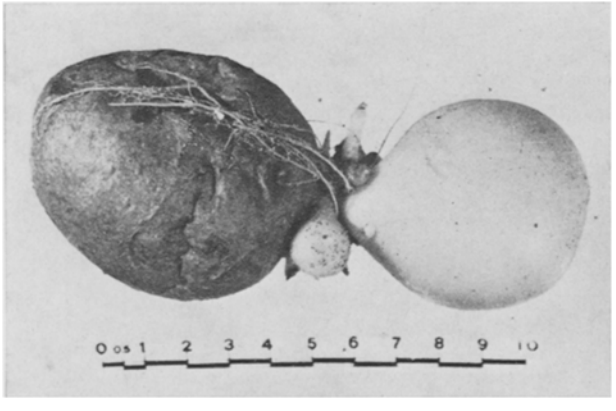

Fig. 3. Tuber formed at 20 °C after 33 days of initiation of tuberization. Normal tubers were produced at this temperature.

This would demonstrate that the effect of high temperatures induces a more rapid incubation state or accelerates the process of physiological age of the seed tubers.

The effect of the state of incubation[11,12] of the seed tuber and sprouts on tuber yield has been shown by tuber growth analysis curves. Plants developed from physiologically old seeds are weak, exhibiting a poor growth and yield.

The notion of the state of incubation reflects more clearly than physiological age the capacity of the potato seed to produce high yields of potato crops; furthermore, the state of incubation is also related to environmental[13–16] factors (temperature, humidity) during storage and also it can be defined by the presence of morphological structures. The great intra-clonal variation of the yield[17,18] is not only due to chronological age but also to other factors which influence the physiological age of the tubers.

It can be concluded that the eco-physiological decline is originated because of the alteration of the incubation state (physiological age) by hastening the senescence of the plants through high temperatures during the formation of tubers that will be used for seeds.

Resumen. En condiciones especiales se indujo la formación de tubérculos sin follaje a temperaturas de 20 °C y 27 °C. Después de la brotación se determina el estado de incubación de los tubérculos formados a ambas temperaturas. Se concluye que la declinación eco-fisiológica de los cultivares de papa se debe a la formación de los tubérculos a altas temperaturas. Las temperaturas de 27 °C inducen un estado de incubación (edad fisiológica) avanzado.

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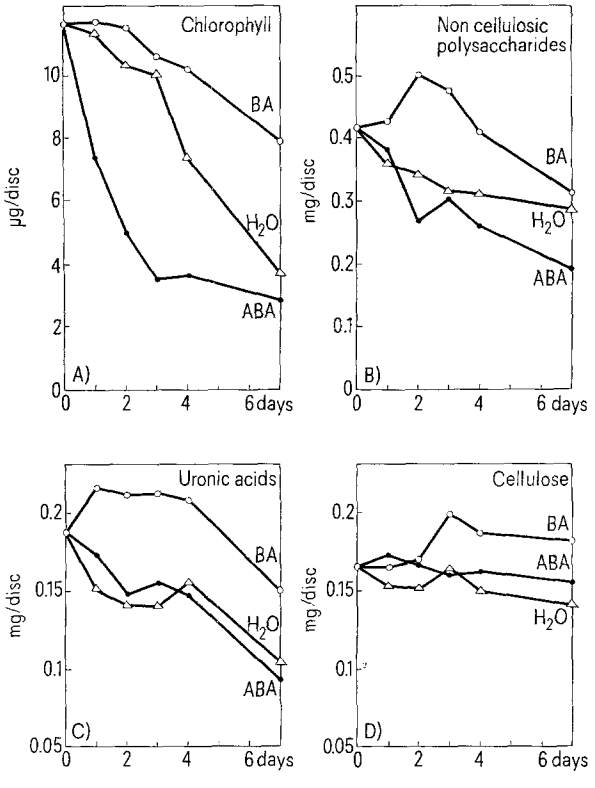
Cell Wall Degradation in Senescing Tobacco Leaf Discs

The involvement of hydrolytic degradation of cell wall polymers in developmental processes such as cell elongation, senescence, abscission, and cell fusion has been demonstrated in various instances (summary in MATILE[1]). In the case of cell elongation, the non cellulosic polysaccharides seem to be chiefly responsible for the dynamism of plant cell walls[2]. In contrast, the surprisingly extensive wall degradation in the senescing corolla of *Ipomoea tricolor* observed by WIEMKEN-GEHRIG[3] comprises hemicelluloses as well as (to a minor extent) cellulose. In this case, the degradation of polysaccharides is most likely brought about by a variety of hydrolases whose activities increase considerably at the onset of wilting[3]. It appears that the rise of enzyme activities and the concomitant lytic events represent phenomena of a precisely regulated process of senescence. Indeed, the hormonal regulation of one of the hydrolases possibly involved in wall metabolism of senescing leaves, a β-1,3-glucanase, has recently been investigated[4]. In senescing leaf discs of *Nicotiana glutinosa*, its activity increases dramatically in the course of incubation; however, the treatment with abscisic acid, a senescence promoting hormone, resulted in a markedly reduced activity of glucanase. Since the corresponding changes of wall con-

stituents in senescing tobacco leaf discs have not been determined, it seemed important to recover this in discs subjected to hormone treatments which are known to alter the course of ageing.

Mature leaves of *Nicotiana tabacum* (cultivar Alta), similar in size and colour, were washed with distilled water. Discs (15 mm in diameter) were cut from the intercostal areas. They were mixed and divided at random into groups of 24, which were floated in Petri dishes on either distilled water, benzyladenine (BA, 10^{-5} *M*) or abscisic acid (ABA, 5×10^{-5} *M*). The upper epidermis of the discs faced the solution, which was changed daily. The incubation was carried out at 27 °C in the dark. At intervals, samples of 10 discs were collected and stored at –20 °C. Cell walls were prepared by first disintegrating the discs in a glass homogenizer in the presence of 0.5 *M* phosphate buffer pH 7.0 supplemented with 10 m*M* ascorbic acid and 0.5% Triton-x-100. Consecutive centrifugations (10 min 2,000 × *g*) and resuspensions (3 times in buffer, 2 times in dist. water) yielded a crude preparation whose starch content was eliminated upon a treatment with pancreatic amylase (0.02% amylase in 0.01 *M* phosphate buffer pH 7.0; 2 h 37 °C). After washings in buffer and distilled water, the non-cellulosic polysaccharides were extracted with 4% (w/v) KOH (3 consecutive treatments, each at 80 °C for 15 min). The KOH-insoluble residue represented the cellulosic fraction. Polysaccharides were estimated using the anthrone reagent[5], uronic acids employing the carbazole reaction[6].

The effect of hormone treatments on the course of senescence appears from the Figure A. As expected, the treatment with ABA resulted in an accelerated yellowing of the discs as compared with the initially slow disappearence of chlorophyll in the control. In contrast, a markedly delayed decrease of chlorophyll characterizes discs treated with the cytokinin BA. These findings are consistent with the results reported from similar experiments with *Nicotiana glutinosa*[4].



Effect of benzyladenine (BA) and abscisic acid (ABA) on senescing tobacco leaf discs. Changes of chlorophyll content (A), noncellulosic cell wall polysaccharides (B), uronic acids present in the noncellulosic wall fraction (C) and cellulose (D). Each point represents the mean value of triplicate determinations.

In tobacco leaf discs, senescence appears to be accompanied by conspicuous changes of contents in non-cellulosic wall constituents. The most pronounced breakdown of these polysaccharides occurs in the presence of ABA: about 50% of the KOH-extractable wall fraction has disappeared after 7 days of incubation (Figure B and C). Since the cellulose content is practically constant in the presence of ABA, the total loss of cell wall mass amounts to about 40%. In contrast, the treatment with BA not only seems to delay the breakdown but results in a slight net increase of hemicelluloses and uronic acid contents during the initial phase of the experiment. Even the content of cellulose seems to increase slightly in the presence of cytokinin.

A preliminary attempt to correlate the observed degradation of cell wall constituents with hydrolytic enzyme activities produced in the senescing tissue has yielded confusing results so far. Of the activities tested in crude homogenates (β-glucosidase, β-galactosidase, β-xylosidase, α- and β-mannosidase; *p*-nitrophenyl-substrates) increases such as observed for β-glucanase in *Nicotiana glutinosa*[4] or in *Ipomoea tricolor*[3] did not occur or the activities were completely absent. High activities of β-glucosidase and α-mannosidase present in the extracts did not change dramatically in the course of senescence. In addition, the lowest of α-mannosidase activities were present in discs treated with ABA in which the most pronounced breakdown of hemicelluloses occurred. A similar effect of ABA on the activity of β-glucanase has been observed in *Nicotiana glutinosa*[4].

Enzyme activities estimated in crude homogenates do not necessarily reflect the hydrolytic potential present in the cell walls. In the present study, the chromophoric, low molecular weight substrates of glycosidases have been chosen because they allow one to assess extra- and intracellular activities separately[1]. The apparent lack of correlation between polysaccharide degradation and total hydrolase activities has, therefore, to be reexamined with regard to hydrolase compartmentation.

Zusammenfassung. In ausgestanzten Blattstücken von *Nicotiana tabacum*, welche auf Wasser schwimmend inkubiert werden, findet im Verlauf von 7 Tagen ein beträchtlicher Abbau der Hemizellulose statt. Benzyladenin (10^{-5} *M*) verzögert den Abbau, Abscisinsäure (5×10^{-5} *M*) verstärkt ihn. Der Gehalt an Zellulose verändert sich im Verlauf der Alterung kaum.

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