CHANGES IN ENDOGENOUS ABSCISIC ACID, SOLUBLE SUGARS AND PROLINE LEVELS DURING TUBER DORMANCY IN *SOLANUM TUBEROSUM* L.

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Abstract

Abscisic acid (ABA), sucrose, reducing sugars and proline contents were monitored over an eleven-month period in tubers of three potato cultivars that varied in tuber dormancy. ABA and proline levels increased after top pulling the haulms and were not affected by harvest date. Proline did not change in either a storage temperature or cultivar-specific manner. The highest concentrations of ABA were found in tubers stored at 2°C while the lowest concentrations occurred in tubers stored at 20°C. At 10°C, the end of tuber dormancy in the cultivars Kennebec and Nooksack (but not Sebago) coincided with the decline in ABA content. There was no evidence of a threshold concentration of ABA below which sprouting would occur. Tuber samples of 10 different cultivars were removed from 10°C and placed in 20°C storage (in darkness). Initial ABA concentrations (*i.e.*, at the time of removal from 10°C storage) were positively correlated with duration of dormancy and negatively correlated with subsequent rates of sprout elongation at 20°C. Sucrose content was negatively correlated with duration of dormancy. Reducing sugars responded primarily to storage temperature and did not appear to be related to dormancy or sprouting.

Resumen

El contenido de ácido abscisico (ABA) sucrosa, azúcares reductores y prolina fueron manipulados por un período de 11 meses en tubérculos de 3 variedades de papa que mostraban una considerable variación en dormancia del tubérculo. Los niveles de ABA y prolina se incrementaron después de defoliar la parte superior de la planta y no fueron afectadas por la fecha de cosecha. El contenido de prolina no cambió al variar ya sea la temperatura de almacenamiento o el cultivar. Las concentraciones más altas de ABA fueron encontradas en tubérculos almacenados a 2°C mientras que las concentraciones más bajas ocurrieron en tubérculos almacenados a 20°C. A 10°C el término de la dormancia en los tubérculos en los cultivares Kennebec & Nooksack (no Sebago) coincidió con disminución en el contenido de ABA.

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No hubo evidencia de una concentración mínima de ABA por debajo de la cual ocurriría brotación de los tubérculos Muestras de tubérculos de 10 diferentes cultivares fueron llevadas de 10°C a 20°C de temperatura de almacenamiento (en oscuridad). Las concentraciones iniciales de ABA fueron correlacionadas positivamente con la duración del período de dormancia y negativamente con las tasas subsecuentes de alargamiento de brotes a 20°C. El contenido de sucrosa mostró correlación negativa con la duración de la dormancia. Los azúcares reductores fueron principalmente afectadas por la temperatura de almacenamiento y aparentemente no mostraron relación con la dormancia o brotación.

Introduction

Recently harvested potato tubers have an internal dormancy which prevents sprout growth from occurring in environmentally favorable conditions. This variable and cultivar-specific dormancy has been shown to be closely related to the content of the acidic inhibitor β complex (7, 9, 10, 11, 12, 13, 14). Studies have indicated that abscisic acid (ABA) is a component of the inhibitor complex and may be one of the main components that prevent sprout growth in tubers during dormancy (16). Additional support for ABA's regulatory role in tuber dormancy is evident in various reports which demonstrate an inhibitory effect by exogenous ABA on sprout growth from non-dormant tubers (3, 6, 25). Although limited evidence indicates that endogenous ABA levels decrease upon the completion of dormancy (16), long-term studies involving different cultivars under different storage environments are lacking and as a consequence, our knowledge of ABA's role in the regulation of the different facets of tuber dormancy is unclear.

The purpose of the present work was to examine the quantitative interrelationships among endogenous ABA, sucrose, reducing sugars, proline, duration of dormancy and sprouting activity.

Materials and Methods

Plant Material and Tuber Handling

During the summer of 1981, seed potatoes were produced from certified stocks of Sebago (a short dormancy type), Kennebec (intermediate dormancy) and Nooksack (long dormancy). Two weeks after top pulling the haulms in early September, tubers were dug and placed in storage at 15°C and relative humidity $\geq 90\%$ for approximately four weeks. Subsequent storage was at 2°, 10° or 20°C. Tubers were sampled at various times prior to and after top pull over an 11-month period for chemical analyses or evaluation of sprouting characteristics at 20°C under continuous darkness. Length of the longest sprout was used to estimate sprout development (26).

In order to evaluate the presence of any significant correlations among the various parameters, tubers from 10 cultivars (Norchip, Tobique, Bintje,

Caribe, Belleisle, Russet Burbank, Shepody, Jemseg, Fundy and Red Pontiac) were removed from 10°C after 2 months' storage and analyzed for ABA, sucrose and reducing sugars. Duplicate tuber samples were placed in a dark 20°C incubator and duration of dormancy (*i.e.*, time to 50% tuber sprouting) and sprout growth rate noted. Correlation analyses and subsequent path analysis (17) were performed on the data.

A separate experiment was carried out in 1982 to determine the effects of top pull date and harvest date on subsequent duration of dormancy in Kennebec. Plants were top pulled on three different dates and three harvests were carried out for each top pull date at two-week intervals. At harvest, tubers were analyzed for proline and ABA content while duplicate samples were placed in a dark 20°C incubator and duration of dormancy recorded.

Extraction and Assay of Sugars and Proline

Six tubers from each sample were chopped finely and used for analysis. Four 1.0 g samples were prepared and analyzed for proline according to the method of Bates *et al.* (2). Two hundred gram samples were prepared and analyzed for sucrose according to the method of Sowokinos (24). Reducing sugars were analyzed according to the method of Lindsay (18). All tubers which had sprouted were de-sprouted before analysis and results were expressed as mg carbohydrate or μg proline per g fresh weight of tuber tissue.

Extraction and Assay of Abscisic Acid

Abscisic acid was extracted in methanol (25 g fresh weight/100 ml methanol) using a modified solvent partitioning method of Milborrow (19). For separation and identification of ABA, a Tracor 222 gas chromatograph equipped with electron-capture ^{63}Ni detector (with linearizer) and a 1.8 m × 4 mm i.d. glass column packed with 3% OV-275 on 80-100 mesh H.P. Chromosorb W was used. The operating parameters were: injection port, 225°C; column, 220°C; detector, 300°C; and 5% methane-argon flow rate, 60 ml/min. Under these conditions, methylated ABA (derivatized by diazomethane) had a retention time of 4.6 min. Internal ABA standards indicated a consistent recovery of 80-90% and the presumptive ABA peaks of the potato extracts were confirmed by GC-MS and peak changes induced by UV-induced isomerization of methylated ABA.

Results

Fresh weight changes during the 11 months were slight except at 20°C where considerable water loss led to an increased percentage of dry matter (Fig. 1).

An examination of "free" ABA levels in Sebago, Kennebec and Nooksack (Fig. 2) revealed that top pulling was important for initiating a pronounced increase in ABA levels. Harvest date had no effect. Low storage temperature led to increased ABA levels. (In Fig. 2, note scale differences in

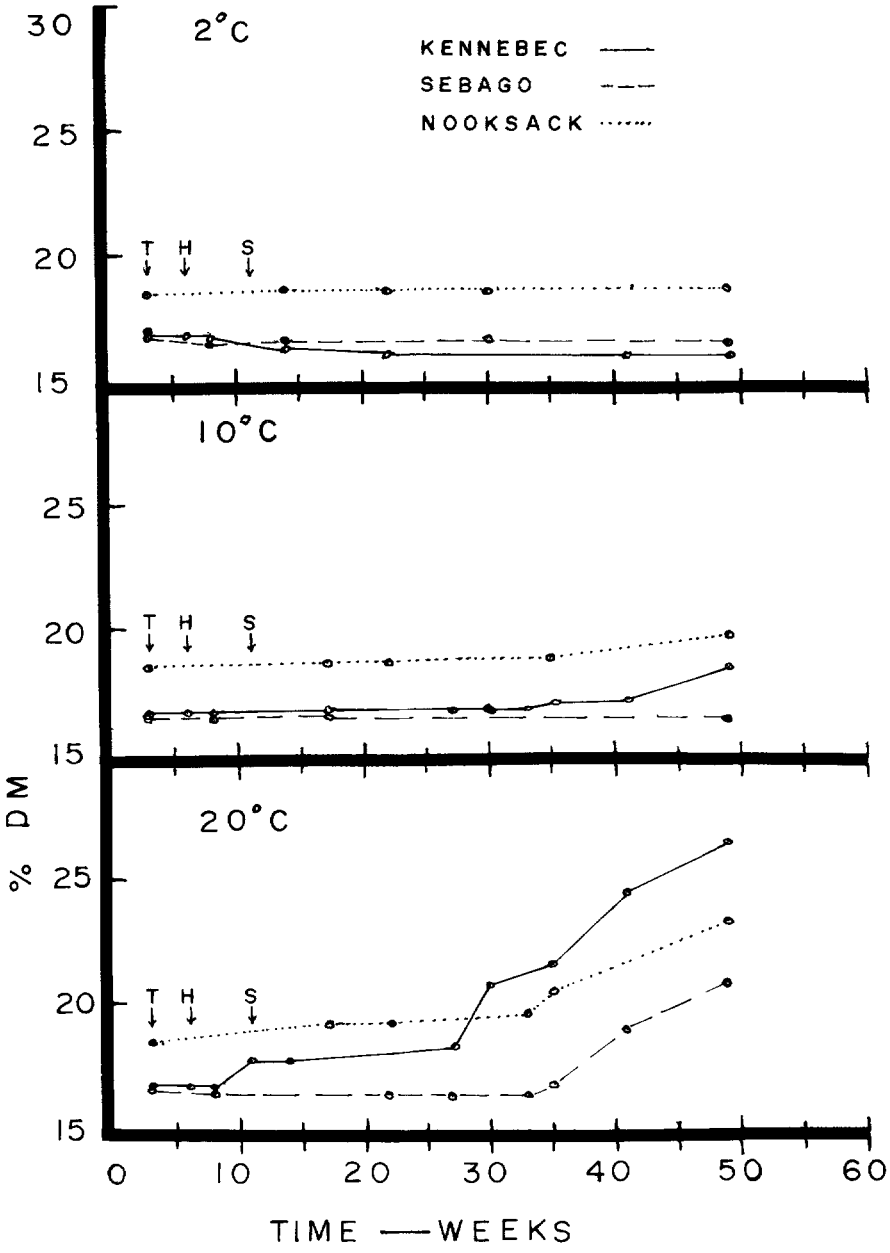

FIG. 1. Changes in percentage dry matter (% DM) during storage of tubers at 3 different temperatures. T = top pull date; H = harvest date; S = tubers removed from 12-14°C "curing" and placed into storage at 3 different temperatures (2°, 10° or 20°C).

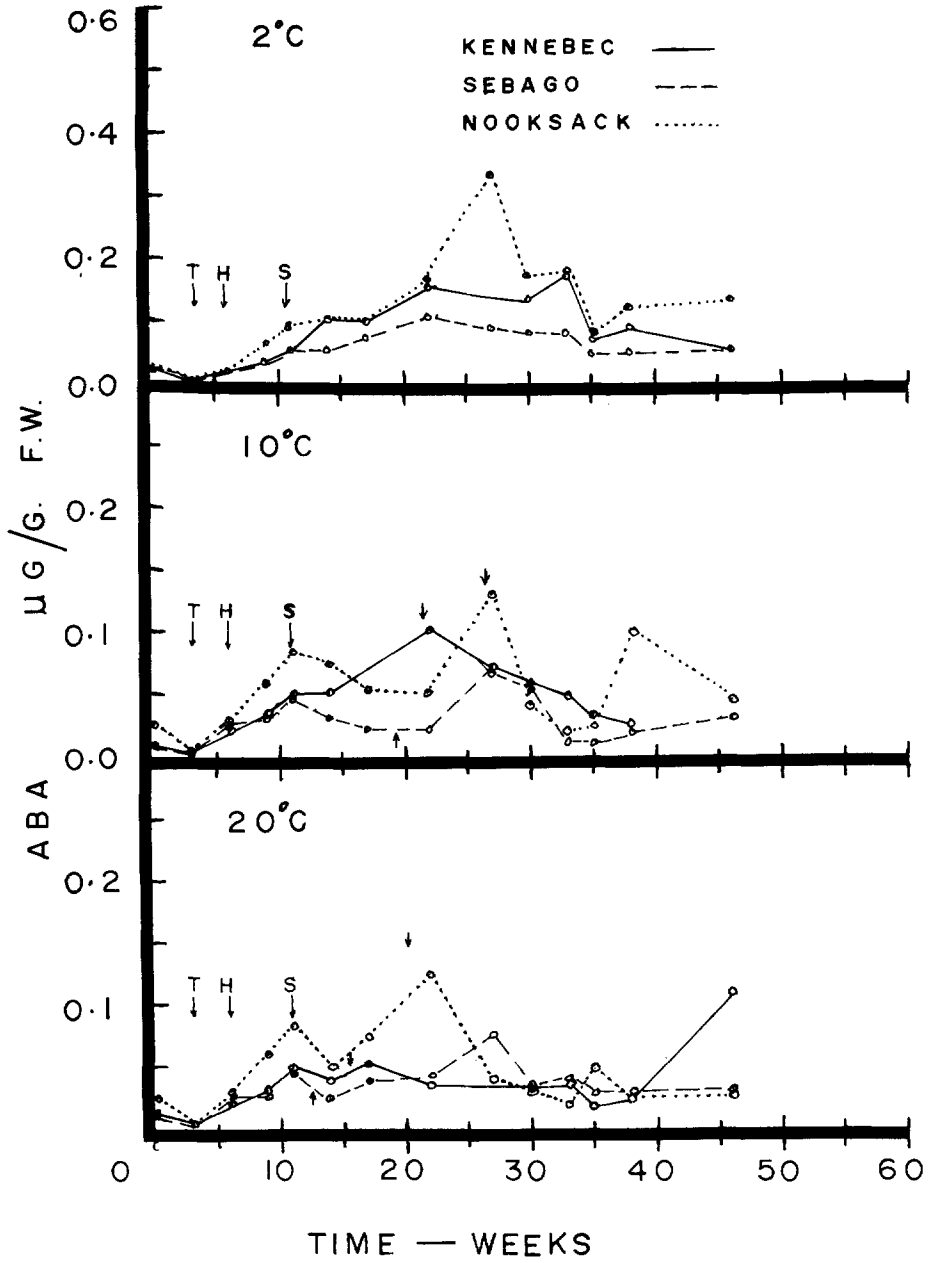

FIG. 2. Changes in ABA during storage. Note ordinate scale for 2°C. Unlabelled arrows refer to the first indication of tubers sprouting (*i.e.*, cessation of dormancy). See Fig. 1 for legend.

ABA content at 2° and 10°C). ABA content at 2°C was positively correlated with duration of dormancy over most of the post harvest and storage period while the initiation of sprouting appeared to coincide with a decrease in ABA

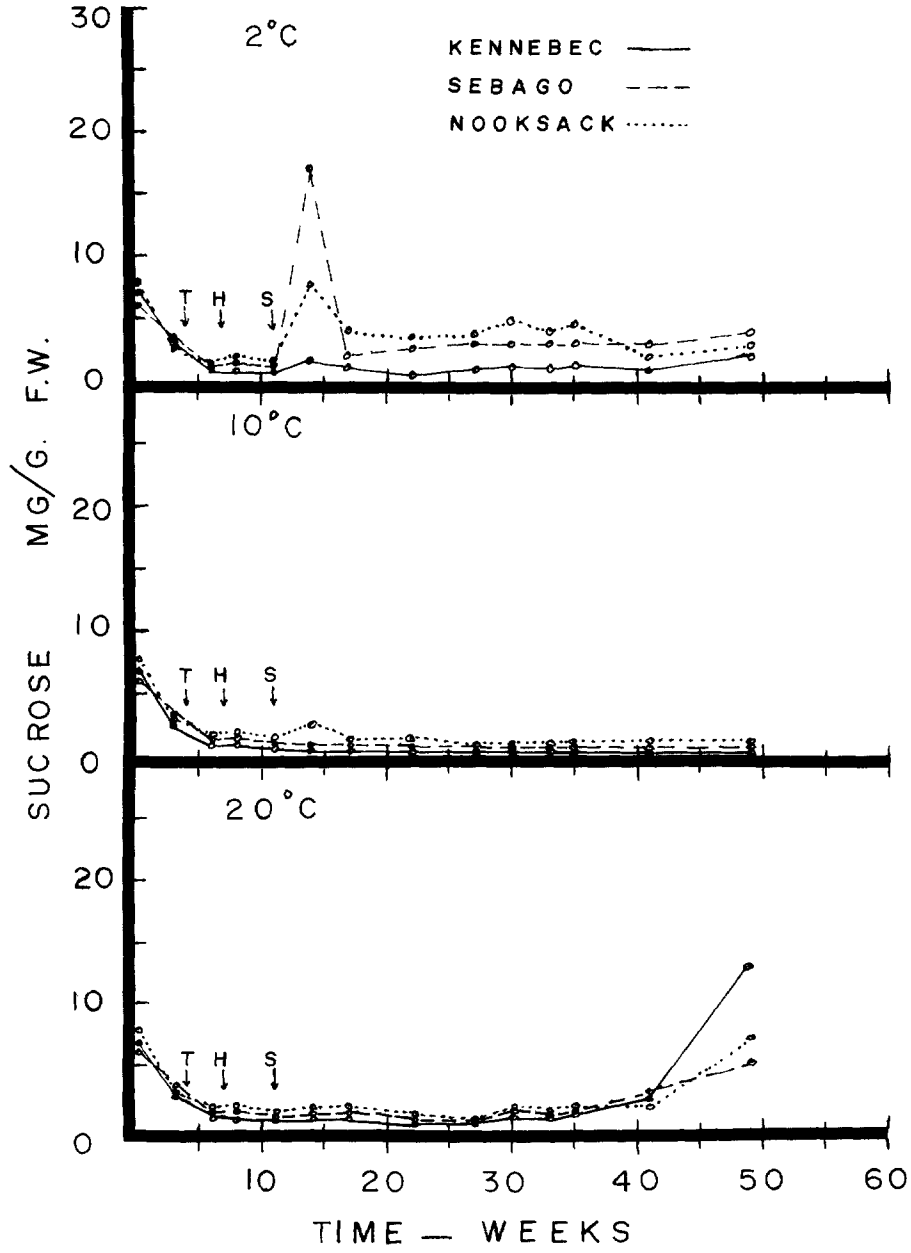

FIG. 3. Changes in sucrose during storage. See Fig. 1 for legend.

content in Nooksack and Kennebec but not in Sebago at 10°C storage. There was no evidence of a threshold concentration of ABA below which sprouting would occur.

Sucrose and reducing sugar levels were primarily influenced by storage temperature in a cultivar-specific fashion (Figs. 3 and 4). "Senescence sweet-

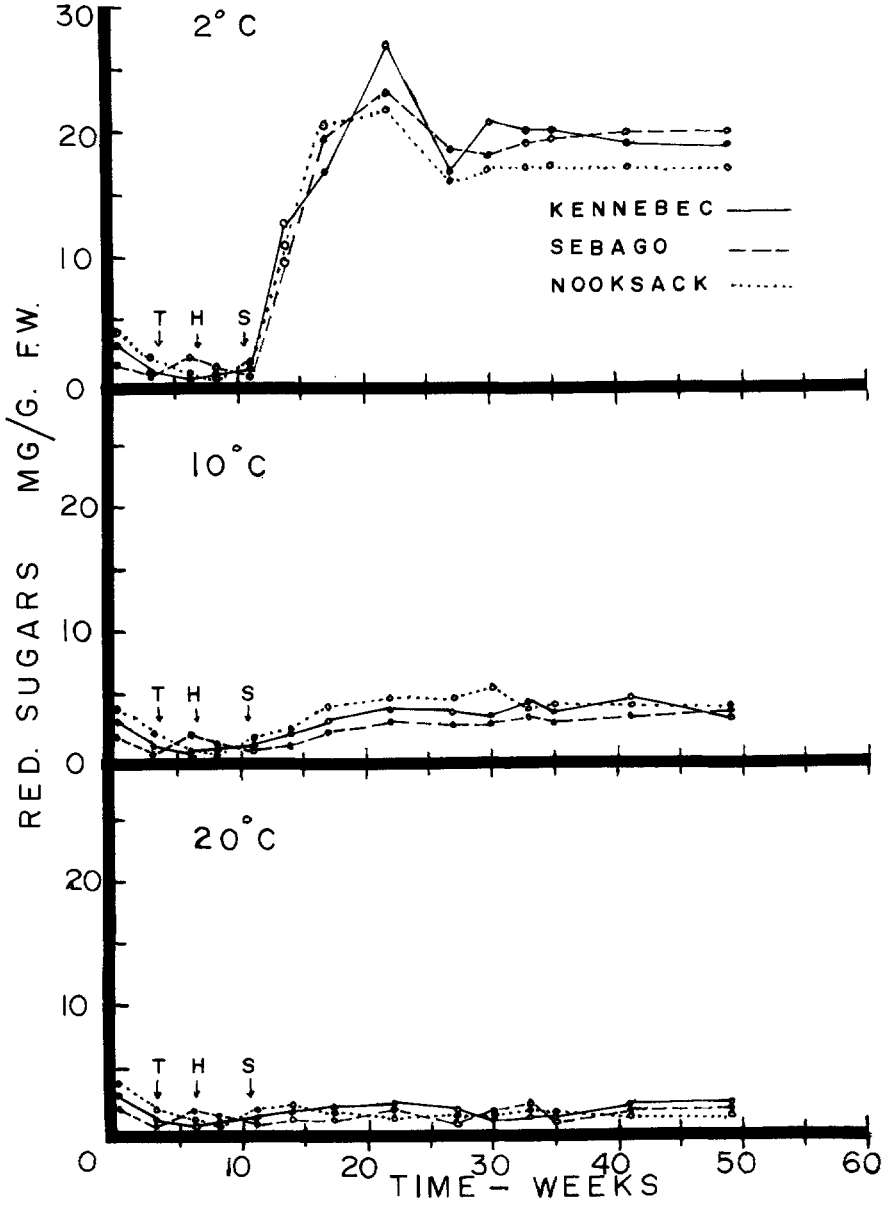

FIG. 4. Changes in reducing sugars during storage. See Fig. 1 for legend.

ening'' (*i.e.*, increasing sucrose content) was evident after approximately 38 weeks' storage at 20°C.

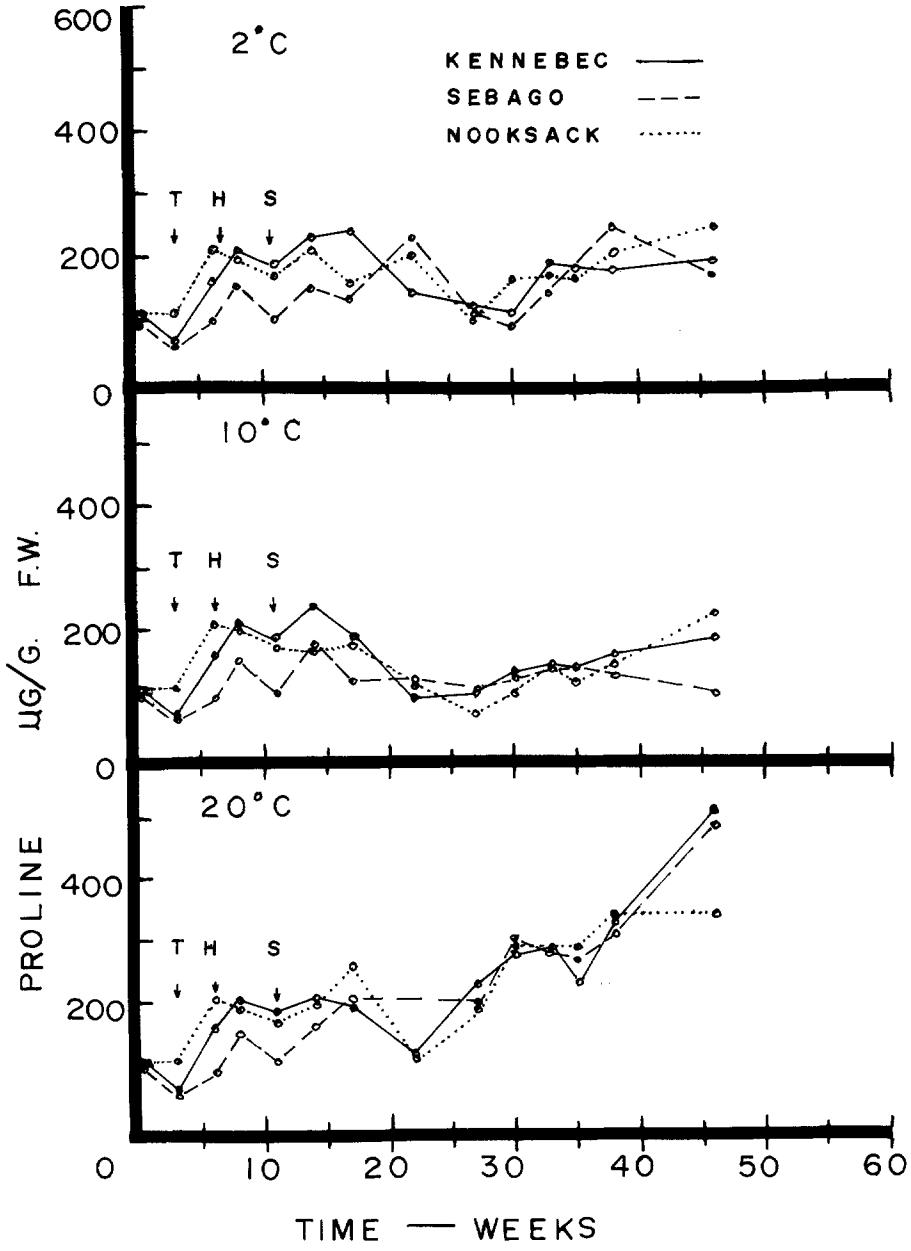

FIG. 5. Changes in proline during storage. See Fig. 1 for legend.

Proline levels changed in the cultivars (Fig. 5) and indicated that top pull date was important for initiating a consistent increase in proline levels. Harvest date had no effect. Low temperature storage (*i.e.*, 2° and 10°C) did not lead to increased proline levels and changes in proline content did not reflect changes in the duration of dormancy or the initiation of sprouting.

An evaluation of the presence of any significant correlations among the various parameters in tubers from 10 cultivars (Table 1) indicated that endogenous ABA levels were positively correlated with duration of dormancy ($r = +.60$) although this result was not significant at the 5% level ($r = +.63$). However, endogenous ABA levels were negatively correlated with subsequent mean sprout growth rate. Additionally, sucrose content was negatively correlated with dormancy duration while the duration of dormancy was negatively correlated with sprout growth rate.

TABLE 1. — *Correlation matrix of ABA, sucrose, reducing sugars, sprout growth rate and dormancy duration for 10 different cultivars after storage for 2 months at 10°C*

	ABA content	Sucrose content	Reducing sugars	Sprout growth rate
ABA content	—			
Sucrose content	−.27	—		
Reducing sugars	−.26	+.75*	—	
Sprout growth rate	−.87*	+.45	+.23	—
Dormancy duration	+.60	−.73*	−.14	−.77*

*Significant at 5% level.

TABLE 2. — *Effect of top pull and harvest dates on tuber dormancy in Kennebec.*

Top pull date	Harvest date	Initial ABA content ($g.g^{-1}$, F.W.)	Initial proline content ($\mu g.g^{-1}$, F.W.)	Duration of dormancy* (days)
July 30	July 30	.022	113.2	125
July 30	Aug 13	.036	124.8	120
July 30	Aug 27	.057	177.6	125
Aug 20	Aug 20	.010	84.4	126
Aug 20	Sept 3	—	200.5	127
Aug 20	Sept 17	.027	156.5	128
Sept 10	Sept 10	.025	149.2	132
Sept 10	Sept 24	.060	225.3	128
Sept 10	Oct 8	.058	172.8	128

*Time to 50% sprouting.

The effects of top pull and harvest dates on subsequent duration of dormancy in Kennebec were studied. Proline and ABA content were also determined at the time of lifting. Harvest date had no effect on dormancy dura-

tion while top pull date significantly affected dormancy duration ($p < 0.10$) when evaluated by the Friedman two-way analysis of variance (22).

When top pull date was delayed by up to 42 days, dormancy was prolonged up to an additional 12 days (Table 2). Top pull and harvest dates had no significant effects on initial proline or ABA contents. However, proline and ABA levels were significantly and positively correlated (+.87, $p < .05$) although they were not correlated with the duration of dormancy or subsequent sprout growth rate.

Discussion

The experimental results described in this paper (see Table 1) can be described in a hypothetical relationship using a "path diagram" (Fig. 6). Although it must be emphasized that no "cause and effect" can be assigned by this method, path analysis is a useful descriptive tool for the development of plausible interrelationships. The path coefficients indicate that sucrose content showed a stronger influence than ABA on the duration of dormancy.

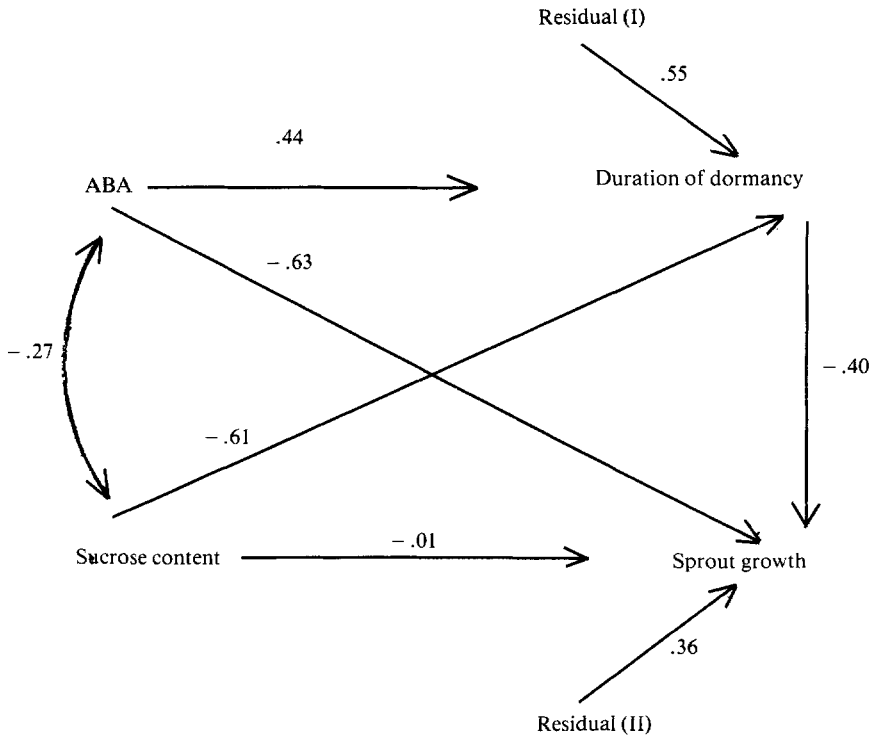

FIG. 6. Path analysis diagram for 10 cultivars showing the hypothetical interrelationships between the different variables and their path coefficients. The double arrow means "correlation" whereas the single arrow indicates hypothetical "causation".

Abscisic acid showed a strong, negative influence on sprout growth, supporting previous experiments in which exogenous ABA inhibited potato sprout growth (3, 6, 25). However, ABA showed a weak, negative influence on sprout growth via duration of dormancy when measured by $.44 \times (-.4) = -.17$.

Sucrose had no direct influence on sprout growth. Rather, it had a weak influence on growth through its apparent effects on duration of dormancy.

The degree of determination of ABA, sucrose content and duration of dormancy on sprout growth is quite high at $R^2 = 87\%$. However, the degree of determination of ABA and sucrose content on duration of dormancy is $R^2 = 70\%$. This result suggests that there must be other factors involved in controlling the duration of dormancy and previous research (*e.g.*, 3, 8) supports this conclusion. Since duration of dormancy is based on visible sprout growth, the dormancy duration may be a secondary outcome of ABA's primary role in the inhibition of sprout growth. Consequently, the existing system does not allow us to distinguish between the two proposed ABA effects. We may have to redefine the concept of dormancy in the potato tuber.

It is possible that both bud and tuber storage tissues exhibit independent dormancy characteristics (1) which would account for the observations that soluble sugar increases can be disassociated from the termination of dormancy and tuber sprouting in response to various chemicals (*e.g.*, 4, 20). Although sucrose content is negatively correlated with dormancy duration (Table 1), the role of sucrose in physiological aging remains to be explored.

The initial increases in ABA and proline content in response to top pulling are similar and are probably indicative of a stress response induced by top removal. The significant positive correlation between these substances (Table 2; $r = +.87$, $p < .05$) during and after top pull may be due to proline accumulation caused by ABA (15, 21). The increase in ABA may also play a role in the suberization phase of periderm development (5, 23).

If ABA is an important factor in the control of dormancy duration and rate of sprout growth, then top pull date may be an important factor in the timing of tuber dormancy since it initiates the ABA increase during the preharvest and early post-harvest periods. The present results (Table 2) indicate that top pull date has a small but significant effect on the duration of dormancy. Previous research has indicated that the time of defoliation is important in the timing of tuber dormancy (27). Further research is necessary to determine whether ABA is the unknown controlling "internal factor" mentioned by Wurr.

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Literature Cited

1. Bailey, K.M., I.D.J. Phillips and D. Pitt. 1978. The role of buds and gibberellin in dormancy and the mobilization of reserve materials in potato tubers. Ann Bot 42:649-657.
2. Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. Plant and Soil 39:205-207.
3. Blumenthal-Goldschmidt, S. and L. Rappaport. 1965. Regulation of bud rest in tubers of potato, *Solanum tuberosum* L. II. Inhibition of sprouting by inhibitor β complex and reversal by gibberellin A_3. Plant and Cell Physiol 6:601-608.
4. Burton, W.G. 1965. The sugar balance in some British potato varieties during storage. I. Preliminary observations. Eur Potato J 8:80-91.
5. Cottle, W. and P.E. Kolattukudy. 1982. Abscisic acid stimulation of suberization: Induction of enzymes and deposition of polymeric components and associated waxes in tissue cultures of potato tuber. Plant Physiol 70:775-780.
6. El-Antably, H.M.M., P.F. Wareing and J. Hillman. 1967. Some physiological responses to d,l-abscisin (dormin). Planta (Berl) 73:74-90.
7. Franklin, J. and T. Hemberg. 1980. Seasonal changes of the different components of the inhibitor complex in potato tubers. Physiol Plant 50:227-232.
8. Hartmans, K.J. and A. Van Es. 1979. The influence of growth regulators GA_3, ABA, kinetin and IAA on sprout and root growth and plant development using excised potato buds. Potato Res 22: 319-332.
9. Hemberg, T. 1947. Studies of auxins and growth inhibiting substances in the potato tuber and their significance with regard to its rest period. Acta Horti Bergiani 14:133-220.
10. Hemberg, T. 1949. Significance of inhibiting substances and auxins for the rest period of the potato tuber. Physiol Plant 2:24-36.
11. Hemberg, T. 1952. The significance of the acid growth-inhibiting substances for the rest-period of the potato tuber. Physiol Plant 5:115-129.
12. Hemberg, T. 1958. The significance of the inhibitor β complex in the rest period of the potato tuber. Physiol Plant 11:615-626.
13. Hemberg, T. 1965. The significance of inhibitors and other chemical factors of plant origin in the induction and breaking of rest periods. Handb Pflanzenphysiol 15:669-698.
14. Holst, U.-B. 1971. Some properties of inhibitor from *Solanum tuberosum* compared to abscisic acid. Physiol Plant 24:392-396.
15. Huber, W. 1974. Uber den EnfluB von NaCl-oder Abscisinsaure-Behandlung auf den Proteinmetabolismus und einige weitere Enzyme des Aminosaurestoffwechsels in Keimlingen von *Pennisetum typhoides*. Planta 121:225-235.
16. Korableva, N.P., K.A. Karavaeva and L.V. Metlitskii. 1980. Changes of abscisic acid content in potato tuber tissues in the period of deep dormancy and during germination. Fiziol Rast 27:585-591.
17. Li, C.C. 1975. Path analysis—a primer. The Boxwood Press, Pacific Grove, California.
18. Lindsay, H. 1973. A colorimetric estimation of reducing sugars in potatoes with 3,5-dinitrosalicyclic acid. Potato Res 16:176-179.
19. Milborrow, B.V. 1972. Stereochemical aspects of the formation of double bonds in abscisic acid. Biochem J 128:1135-1146.
20. Miller, L.P. 1933. Effect of various chemicals on the sugar content, respiratory rate and dormancy of potato tubers. Contrib Boyce Thompson Inst. 5:213-234.
21. Rajagopal, V. and A. Skytt-Andersen. 1978. Does abscisic acid influence proline accumulation in stressed leaves? Planta 143:85-88.

22. Siegel, S. 1956. Nonparametric statistics. McGraw-Hill, New York.

23. Soliday, C.L., B.B. Dean and P.E. Kolattukudy. 1978. Suberization: inhibition by washing and stimulation by abscisic acid in potato disks and tissue culture. Plant Physiol 61:170-174.

24. Sowokinos, J.R. 1978. Relationship of harvest sucrose content to processing maturity and storage life of potatoes. Am Potato J 55:333-344.

25. Van Es, A. and K.J. Hartmans. 1969. The influence of abscisin II and gibberellic acid on the sprouting of excised potato buds. Eur Potato J 12:59-63.

26. Wurr, D.C.E. 1978a. Studies of the measurement and interpretation of potato sprout growth. J Agric Sci Camb 90:335-340.

27. Wurr, D.C.E. 1978b. 'Seed' tuber production and management. *In:* P.M. Harris (Ed.), The potato crop, the scientific basis for improvement, pp. 327-354. Chapman & Hall, London.