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FACTORS INFLUENCING THE INITIATION AND
RATE OF SOLANINE SYNTHESIS IN TUBERS
OF SOLANUM TUBEROSUM L.

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April, 1955

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ABSTRACT

Investigations were undertaken to determine environmental and varietal influences upon the concentration of the bitter and poisonous components, solanine and solanidine, in tubers of Solanum tuberosum L.

Soil types, geographic locations and the photoperiod applied to growing plants did not directly affect the glycoalkaloid content of the tuber. Varieties differed markedly in the amount of bitter component they were able to synthesize, and in the external conditions at which they built up solanine most actively. They have been classified into three divisions: (a) those not subject to high concentrations under any conditions; (b) those capable of marked increase but not to levels of human toxicity (200 p.p.m.); and (c) those that readily reach dangerously high levels when environmental conditions are such as to encourage this synthesis.

An important observation that refutes reports of other workers is that all varieties consistently contain low concentrations of solanine at harvest time; but most are able to develop the alkaloid rapidly (and convert it to the glycoalkaloid) if exposed then or later to solar or artificial illumination, even if the exposure is of but a few hours' duration. The rate of synthesis is greatest in the earliest stage of ex-

posure period. Also, some varieties (in particular, Netted Gem) will develop solanine from a threshold to a high concentration at a steady rate in storage at 48° F. or less. The normal solanine content of Netted Gem tubers when harvested varies from 20 to 60 p.p.m., but under favourable conditions has been found to reach above 800 p.p.m.

The free alkaloid solanidine was determined by difference between colorimetric and hydrolytic methods of estimating solanine. Contrary to previously reported research, solanidine was found to be present in bitter tubers, in amounts up to one-half the total bitterness component. A low respiratory rate in tubers in the presence of light produces solanidine first, some or all of which is later converted at a relatively steady rate to the glycosidal form. The peel of bitter tubers contains about one-third the total tuber solanine, and the peel concentration is six times that of the flesh.

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FACTORS INFLUENCING THE INITIATION AND RATE OF
SOLANINE SYNTHESIS IN TUBERS OF
SOLANUM TUBEROSUM L.

A THESIS

submitted to the School of Graduate Studies
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INTRODUCTION

An investigation into conditions that may lead to an abnormally high synthesis of solanine in tubers of Solanum tuberosum L. was suggested by an increasing number of complaints from potato growers and consumers concerning unpalatable and bitter-tasting table potatoes. The bitter component is solanine, a glycoalkaloid possessing poisonous properties and normally present in all potato tubers in very small amounts.

With attention drawn to the occurrence of this disorder in our most important vegetable staple, several municipal health officers and other medical practitioners became concerned about the influence that consumption of bitter potatoes may exert upon human health. In some cases (1954 and 1955), the opinion has been expressed that numerous cases of unexplained sickness may be caused by solanine poisoning. In view of the specific nature of complaints, and the close relationship of symptoms to those recorded for solanine poisoning, it is certainly possible that the incidence of potato bitterness is more serious than has been realized hitherto.

Despite the fact that the occurrence of abnormal and toxic solanine concentrations in bitter potato tubers has been known for some fifty years, the knowledge of natural conditions

leading to the disorder has been somewhat fragmentary and confined to many contradictory statements and inadequate observations, partly due to a lack of reliable analytical procedures, and partly to the sporadic nature of "bitter potato" outbreaks, and their localized nature with respect to the geographical areas involved.

With regard to the present investigation, it was felt that a detailed knowledge of conditions and factors responsible for initiation and the rate of solanine synthesis in the potato tuber was an essential prerequisite to the development of remedial measures.

Previous research at the University of Alberta has established that certain environmental factors play an important role in the development of bitter potatoes, and has provided some information concerning the extent to which bitterness may develop. The present work was undertaken to obtain further elucidation of the influences on this component of pre- and post-harvest conditions of environment and of differences that might be expected as a result of heredity.

LITERATURE REVIEW

The botanical and cultivated forms of Solanum and Lycopersicon genera produce a variety of glycoalkaloids. These are basic substances poisonous to warm-blooded animals and closely related to sterols, and they occur naturally in the form of glycosides (24, 45, 50). Extensively studied glycoalkaloids of solanaceous plants include solanine (S. tuberosum L.), demissine (S. demissum Lindl.), tomatine (L. esculentum bacc. ent.) and solasonine (S. sodomium L.), and there is reasonably good agreement among research workers concerning the general nature of their structural configuration and chemical properties, (50). The glycoalkaloid solanine was discovered as early as 1820 by Desfosses in S. nigrum L. (cit. 14). Because of its toxic properties, it soon became the subject of extensive investigation when it was found by Baup to occur in sprouts of the cultivated potato (S. tuberosum L.) (cit. 50).

Chemical and Physical Properties of Solanine

These have been studied by numerous workers, and the structural formula configuration now considered correct is presented in Fig. 1. On acid hydrolysis, solanine yields one molecule each of rhamnose, galactose and glucose, and the basic aglycone solanidine, $C_{27}H_{43}ON$ (12, 61). The steroid nature of solanidine was established by selenium degradation/^{to} γ -methylcyclo-

Figure 1. Structural configuration of the glycoalkaloid solanidine.
 After Briggs and Vining (13), Prelog and Szpilvogel (43),
 Uhle and Jacobs (58).

penteno-phenanthrene, a characteristic dehydrogenation product of the sterols (24, 50). The structure of the basic portion containing one atom of nitrogen has been a subject of many controversies and speculations (13, 53), until the correctness of the formula of Prelog and Szpilvogel (42) was proven by partial synthesis of allosolanidan-3(β)-ol from sarsapogenin by Uhle and Jacobs (57). Additional evidence confirming the steroid nature of the glycoalkaloids was obtained by the preparation of derivatives from structurally ascertained sterols, and vice versa by obtaining these compounds as a degradation product of the alkaloid. Thus Uhle was able to synthesize solasodine from non-nitrogenous kryptogenin (56), and Briggs and O'Shea succeeded in converting solasodine to diosgenin, a sapogenin (11).

Physiological studies on the development of solanine in plant material have been difficult to evaluate because of discrepancies introduced by the lengthy analytical procedures used. The inaccurate gravimetric procedure of Bomer and Mattis (5) was replaced by a more sensitive and precise method involving colorimetry (41). Also, in the extraction of plant material, many improvements and modifications culminated in the relatively short and more accurate technique reported by Dabbs (15, 16). The hydrolytic method of Conner (14) is valuable as an aid in the estimation of solanidine, although relatively high concentrations of glycoalkaloid in the potato tuber are needed before it can be rated as reasonably accurate.

Solanine is present in numerous tuberous Solanum species, although the findings of early workers (50) need to be corroborated. The natural occurrence of free solanidine is said to be rare, and although found in sprouts of some potato varieties (13, 33), it is suggested that it arises by hydrolysis of solanine during the preparation of plant material or during the acid-extracting procedure (50).

In plants of the cultivated potato, the highest glycoalkaloid concentrations are found in sprouts (0.4 to 0.7 percent of fresh weight) and flowers (0.2 to 0.4 percent), the lowest concentrations being found in stems (0.003 percent) and tubers (up to 0.012 percent) (33). Within the tuber itself, normally most of the solanine is concentrated in the eyes and peels, while the flesh contains relatively small amounts. According to Fischer and Thiele, most of the solanine is found in about a ten-cell layer beneath the skin (20).

Solanine and Potato Bitterness

Marked interest in the condition leading to abnormal solanine formation was aroused during the first World War when 61 cases of alleged potato poisoning were reported in Scotland. One of these cases was fatal, and the responsible lot of potato tubers was found to contain 410 p.p.m. of solanine (23). Similar reports came from Germany in 1922 and 1923 (6, 21, 49), and today there is no doubt that tuber bitterness and pharynx irritation from potatoes are due to somewhat larger-than-normal amounts of

solanine (25). Recently, the consumption of potato berries caused the death of a two-year-old girl after 13 days (48). In Alberta, reports of bitter potatoes are increasing as the public is becoming aware of the danger connected with the ingestion of such potatoes.

Potato tubers with a high solanine content leave a bitter and persistent burning sensation in the throat (25). Solanine, which resembles saponins in physiological action, is described as a protoplasmic poison and potent hemolytic, causing headache, nausea and gastritis (24). About 0.22 mgms. of solanine will hemolyze 0.1 ml. of sheep erythrocytes (32). From other alkaloids, solanine differs by its strongly irritating effect on gastro-intestinal mucosa. This effect tends to lower its toxicity, since large doses cause vomiting and diarrhea, thus removing much of the solanine before it can affect the central nervous system (48). The alleged toxic level of 200 p.p.m. for humans that has been reported by Bomer and Mattis (6) is generally agreed upon by other research workers who have investigated this phase of the problem.

Solanine Studies - Historical

As early as 1907, von Morgenstern found high solanine tubers to have a bitter and acrid taste. His pioneering work was concerned with solanine contents of European potatoes (37)

and was followed by the extensive studies of Bomer and Mattis, also in Germany (6, 7), Lampitt et al in Great Britain (33), and Wolf and Duggar who carried out a solanine content survey of American potato varieties (59). The study of solanine and related glycoalkaloids has received much attention of Russian workers (40, 43, 44, 51), where the problem of bitterness in such an important vegetable staple is apparently of great concern (51). Since 1950 a research program has been underway at the University of Alberta (15, 25, 27, 62), principally directed towards improvements in the accuracy and speed of analytical methods, and an investigation into causal factors and the mechanism of synthesis.

In spite of the fact that some 160 research papers have been published that deal with one or more phases of the potato bitterness problem, this number is not great when one realizes that these reports represent all that has been done during the last 130 years. A general impression gained from a study of the literature is that the achievements in the field of determination, of causal factors, and of the physiological plant conditions conducive to high synthesis of the glycoalkaloid, have been of an indecisive and often confusing nature. It does appear that the sporadic nature of research interest is due largely to the fact that severe bitterness in the potato crop occurs only at irregular intervals - such as the "high solanine years" that have occurred in Alberta in 1949 and 1954. Moreover, until

an improved analytical procedure was recently developed, as reported by Dabbs and Hilton (16), the methods of extraction and determination of solanine were very time-consuming and subject to extensive error.

Literature Relating to Solanine Synthesis in Plants and Tubers

The solanine content of potato plants and tubers is highly variable and apparently is governed by three principal factors: inheritance of varietal ability to synthesize large amounts (or inability to maintain low levels); stage of development (strong vegetative growth, tuber maturity, rest period and resumption of physiological activity); and conditions of the environment.

(a) Potato plants

Little of a positive nature is known about solanine transformation and translocation in the growing plants, except statistical data concerning its distribution in various parts of the plants (33, 37) and the fluctuations within major plant organs during the growing season (59). The means whereby glycoalkaloid synthesis occurs in the plants are not well understood, although there is evidence that the site of synthesis is in the leaves and not in the roots (50). Since the Belladonna alkaloids and nicotine are reported as being synthesized only in the roots (29, 30), it appears that the pathway of Solanum glycoalkaloid synthesis is different. Biochemical studies of the mechanism of alkaloid

synthesis in the plant leaf, and of the possible functions of these compounds, are delayed by reason of a lack of basic information concerning the precursory substances and the enzymatic systems involved. Although the existence of a solanine-hydrolyzing enzyme, solanase, in young potato sprouts was shown by Petrochenko (40), nothing positive is known about possible precursory metabolites or processes related to the synthesis. It is likely that some amino acids are involved, as indicated by the need of arginine and ornithine in the synthesis of Belladonna alkaloids (7, 29). Increased solanine formation in greened tubers (6, 34, 37) leads to the assumption that photosynthesis and/or respiration may be closely related processes in providing the required energy or building material for the synthesis.

The function of glycoalkaloids, as well as of alkaloids, is obscure, and they are generally dismissed as secondary by-products of metabolism (7). Nevertheless, factors such as their presence in places of high metabolic activity (33), the increased rate of synthesis in the early stages of plant development (39, 59), and the apparent destructive metabolism that takes place towards the end of the season (37, 59) point towards some constructive reason for their existence. As some glycosides have been shown as controlling factors for self-sterility in Forsythia genus, depending on the presence or absence of the appropriate enzyme (31), the glycoalkaloids may participate at least in certain developmental stages in a vital process of

a similar nature. It is known that free solanidine appears in tuber sprouts of certain potato varieties (13, 33), and that the alkaloid content in sprouts increases with a parallel decrease in the tubers (33).

(b) Potato tubers

The amounts of solanine in tubers vary widely with respect to variety, stage of development and the environment. This variability appears to be dominated by a complex interaction of internal and external factors.

Numerous studies on solanine in American (59) and European (6, 37) potato varieties indicate that concentrations often described as "normal" appear to be determined by varietal character. The general range is from 20 to 130 p.p.m. of fresh tuber weight. In a recently published work dealing with 58 European varieties, Lepper reports values exceeding 200 p.p.m. in about seven percent of the samples; and though the extent of variation within a variety is not shown, it was also stated that high solanine development appears to be a characteristic of some varieties (35). Such relatively high values do not appear as "normal," considering the occasional extremely high concentrations exhibited by some varieties which have been attributed to certain effects of the environment as very low levels of solanine are displayed at maturity in the same varieties if such effects can be excluded (62). The inheritance of the glycoalkaloid synthesis

 

the twenty-fifth is the fact that the

(or amounts) in potato varieties is discussed by Prokoshev et al, who have shown that inter-species hybrids of Solanum contain glycoalkaloid concentrations that are intermediate between the content of parent plants (44), though it is not known whether it was the contents of plants or of tubers that was determined.

In the early tuberization stage, potatoes exhibit higher concentrations, which decrease towards maturity (39, 59), at which time larger tubers appear to have less solanine than small ones (6). The latter observation probably related to the fact that the small tubers have relatively more skin surface than large tubers, and the solanine content is greatest in or near the peel (20). Green and sunburned tubers are often bitter and contain larger-than-normal solanine amounts (6, 14, 37).

(1) Growth conditions

Numerous studies of growth conditions and their possible effects upon solanine formation in mature tubers seemed to yield negative results insofar as the factors responsible for bitterness were concerned. Although reports are somewhat contradictory in many respects, none of the pre-harvest conditions appeared to be effective in inducing an abnormal synthesis, with the exception of situations where sunburn and consequent greening of tubers occurred (34, 37). The presence of chlorophyll is not necessarily an indication of tuber bitterness, as samples of

Alberta-grown potatoes with absolutely no discernible pigmentation were found to have exceedingly high concentrations (27).

The influence of cultural practices such as depth of planting, level of hilling, and planting of single or multiple-eye seed-pieces proved to be negligible (27, 28); as also did various pre-harvest treatments such as chemical vine-killing, and mechanical removal or damage of vines (62). Considering the fact that high solanine tubers as "seed" will produce tubers with normal alkaloid content within one (62) or two (58) years, it appears that normally developing plants possess mechanisms to maintain such low levels in tubers and also to prevent translocation from the solanine-rich tops (59).

In spite of an increase of nicotine (19) and Cinchona (36) alkaloids attributed to the application of high fertilizer rates, similar work with potatoes yielded no conclusive evidence. Levels of solanine seem to be influenced by high dosages of nitrogen and potassium in the early tuberization stage, but the effect is not reflected upon the alkaloid content of mature tubers (39). Von Morgenstern found an increase of tuber solanine with nitrogen and phosphorus fertilizer application (37), while other authors maintain that the concentrations are unaffected by higher rates of N, P, K (6, 34, 49).

Reports on the effect of soil and climate are rare and inconclusive, but it is claimed that when tubers are produced

under conditions of hot climate and dry, sandy soils, more solanine is likely to be produced (2).

(2) Harvest conditions

In view of the doubtfully important influences of plant growth conditions on tuber solanine development, it appears that abnormal formation takes place either at harvest or during subsequent storage. Immature tubers are thought to have higher concentrations (6), but this may be due primarily to smaller tuber size (59), and such evidence is indefinite in view of the alkaloid decrease towards the end of growth (29, 37) and the very low concentrations found in tubers harvested about a month prior to usual harvest time (62). The suggestion that freezing temperatures just prior to harvest may cause a rise in solanine (27) could not be confirmed (62).

Marked solanine formation was observed on exposure of freshly harvested potatoes to solar radiation, and the rate of synthesis appeared to be influenced by the variety and temperatures (62).

While some workers found no appreciable changes during the storage (17, 37, 59), others report an increase (20, 33, 62), or even a decrease (34, 38); however, most of them agree that there is a sharp rise in solanine levels just prior to sprouting (38). The contradictory nature of the findings probably is due to a differential response of varieties under varying storage

conditions (62), as Arutyunyan reports no change at -6° to -8° C. (2), and Naumov reports a decrease at 5° to 7° C., which amounts to 50% when tubers are washed before storage (38). Organoleptic tests also seem to indicate that less bitterness is accumulated or maintained at temperatures above 10° C. (25).

Differences between active and dormant potatoes were also studied, but did not yield conclusive results (14), except that in the early phase of dormancy solanine levels appear to be more readily influenced (62).

Numerous studies were carried out on the effects of light and of specific spectrum components upon solanine synthesis. Schowalter and Hartmann did not believe that light effects may be responsible for abnormal synthesis (49). Conner found the ultra-violet light at about 300 m μ wave-length effective in increasing solanine levels, but wave-lengths effective in glucose synthesis did increase chlorophyll but not solanine (14). Although infra-red light was ^{not} considered by this author to be an effective factor, it was shown that irradiation with an infra-red light source of shorter wave-length than used by Conner induces abnormal synthesis of the glycoalkaloid in dormant tubers. This is accompanied by chlorophyll formation and later in the storage by the appearance of purplish pigments resembling anthocyanins (62). Lepper noted, on exposure to light, an increase in tuber greening and the development of bitter tubers with large amounts of solanine. The solanine content was reduced during the sub-

sequent storage (34). Bomer and Mattis observed increases in solanine in "young tubers" with or without attached tops when these were exposed to light for 7 to 10 weeks, while "old tubers" remained unaffected (6).

From the foregoing, it is seen that exposure to light is considered by the majority of workers as an effective means of inducing solanine synthesis. Little information, however, is given concerning the parallel influence of other factors (temperature, light intensity, varietal response and metabolic state), which recently have been shown to exert a profound influence upon light-induced solanine synthesis (62). Considering the fact that solanine is also synthesized in darkness in sprouting potatoes (49), it appears that the glycoalkaloid occurrence is not dependent upon any single factor of the environment, and that it takes place naturally as a result of a resumption of metabolic activities at the end of the post-harvest rest period. For abnormal synthesis, such as may be found in bitter tubers, it is probable that a photochemical induction stimulus is required.

STUDY OBJECTIVES

Four experiments were designed to investigate the conditions which could lead to abnormal rates of solanine synthesis in tubers. A fifth trial was undertaken to obtain information upon the occurrence and distribution of solanine and solanidine in various tissues of bitter tubers. Observations reported in earlier work suggested approaches along the following lines:

I. To study the influence of representative soil types upon solanine concentrations of mature tubers and to seek additional evidence relative to the hypothesis that abnormal synthesis rarely occurs before the tubers are harvested (62).

In order to include possible influences of important soil types, experimental plots were planned for three sites, widely differing with respect to soils. Confirmation of low solanine concentrations in mature non-pigmented tubers would imply that abnormally high solanine in bitter tubers cannot be due to the translocation from tops, but rather is a result of post-harvest conditions to which tubers are exposed, interacting with the presumably inherent ability of some varieties to produce relatively large amounts of the glycoalkaloid.

II. To study the effects of continuous and intermittent exposure of potato tubers to sunlight under harvest-time conditions, particularly in relation to temperatures normally encoun-

tered during that period.

The activating nature of light in the synthesis of excessive amounts of solanine has been demonstrated and acknowledged for many years (6, 14, 21, 33, 34), although it is also known that in developing sprouts solanine can be synthesized in darkness (49). Recently, the effectiveness of light was shown to be influenced by the temperature and the metabolic state of stored tubers (62). A rapid rise in solanine levels has been noted in a limited number of samples after very short exposures of mature tubers to sunlight. In order to establish adequate statistical evidence for this result, the investigation was extended to eight varieties and^a much greater number of samples. The effect of three soils was included as an additional variable; and in order to establish whether the effect of growth conditions or responses may have caused a delay in glycoalkaloid development, the solanine levels of exposed tubers were determined after an eight-week storage period.

III. To investigate the effects of short and long growth photoperiods on solanine development in tubers.

The suggestion that higher rate of solanine translocation may occur under a shorter photoperiod (59) implies that a decrease of radiant energy during unfavorable growing conditions, as during periods of excessive rain and cloudiness, may result in excessive amounts of solanine in mature tubers. Such a finding

would provide evidence somewhat contrary to the results sought in the first experiment, but it might explain the incidence of "solanine years" as a result of climatic conditions. Such years were reported in Scotland in 1917 (23), in Germany in 1922 and 1923 (6, 49), and have been observed in Alberta, particularly for the 1954 potato crop.

IV. To study fluctuations of solanine levels in stored mature tubers in the presence or absence of light.

Contrary to repeated conclusions that little metabolic change takes place during the storage of potatoes, a slow increase in solanine concentrations was recently reported for stored samples of Netted Gem variety, and the amount of alkaloid approached the alleged toxicity level after continuous illumination for seven days (62). The differences observed in the earlier trial in varietal responses, and the suspected dependence of the illumination effect upon the temperature of the storage chamber, could not be subjected to biometrical treatment. A new trial was therefore designed that would allow statistical analysis of results, and more precise information concerning the influence of the illumination interval on the rate at which solanine synthesis was initiated.

V. To establish the presence or absence in bitter tubers of the free alkaloid; and the distribution within the tubers of solanine and solanidine.

Although solanidine is reported to occur in the free state in certain tissues, the possibility that it may also occur in bitter potatoes has received some negative comment and, in general, little attention. The widespread occurrence of bitterness in the Alberta 1954 potato crop, with record high total solanine concentrations, made it appear desirable to determine the presence or absence of the free alkaloid and the distribution of total solanine within bitter tubers. The study was facilitated by the discovery of a method by which exceedingly high levels of bitterness are produced in susceptible varieties.

FIELD AND SAMPLING PROCEDURE

Eighteen potato varieties were grown in identical experimental plots located in three major Alberta soil zones. Tuber samples from these plots supplied analytical material for the major phases of the work reported here. Some additional material, including Netted Gem and Katahdin varieties, was grown in an experimental plot at the University of Alberta wherein treatments for observation of photoperiodic effect were made, and from which tubers were obtained for exposure to light and to storage in darkness.

The three "variety" and "location" test plots were planted in a randomized block design at the following locations:

(1) the Provincial Horticultural Station at Brooks, in the brown soil zone; (2) the University of Alberta trial grounds, in the black soil zone at Edmonton; and (3) leased farmland at Fallis, in the gray-wooded soil zone.

All tubers used for analytical purposes, except those used in Experiment V, were grown during the 1953 season. The three plots were laid out in identical fashion, even to the row direction. Each consisted of 20 rows at three feet apart, including single guard rows on the two sides. Four blocks, 12 plants in width, were separated by two guard plants planted across the rows. Each variety was represented by a 12-hill row, with seed-pieces planted at 12 inches, and its location was determined by randomization in each block. The total number of experimental plants at each site was 1080. Planting was done between May 27 and June 1, 1953; hilling and the first roguing for virus infection took place in mid-July. Virus disease was responsible for the complete removal of three varieties, prior to the harvest. These three are of recent and similar genetic origin, and are known under number only as 50-1, 50-2 and 176. The other 15 varieties that were harvested for tuber samples are as follows:

Canus	Katahdin	Warba
Carter's Early Favorite	Latowski	C-16
Early Dewey	Manota	177
Early Ohio	Netted Gem	50-3
Irish Cobbler	Penner's Blue	50-7

Plants in all plots grew well in spite of the season being unusually cool and wet. Harvesting was done between

September 30 and October 6; the growing days for plants at Brooks being 122, at Fallis 130, and at Edmonton 133. Rain delayed the harvest at the latter stations. The yield was normal from the black and the brown soil areas, and relatively light from the gray-wooded soil. In all cases, however, satisfactory sampling could be done on a single-hill basis. The weights of all samples for storage studies were taken at harvest, but no other yield data were recorded.

The additional Edmonton plot grown during 1953 was made up equally of Katahdin and Netted Gem varieties, planted in such a manner as to allow freedom in superimposing the light-excluding cage over plants of both varieties.

Method of Sampling

Tuber samples for solanine analysis were taken on the basis of single-hill plants.* Hills were chosen at random, the plants of which were vigorous and healthy in appearance, and the tubers were harvested and placed in marked paper bags and immediately under a covering of potato sacks, to prevent undesirable temperature and light effects. At the time the samples were macerated for frozen storage, four to six healthy and uniform 100 gm. tubers (medium size) were selected and the remainder discarded. Particular care was taken to avoid over or under-sized tubers, and also any tubers showing traces of chlorophyll pigment-

* See Appendix VII for additional notes concerning field and sampling technique.

ation. All samples were brought to the laboratory, where they were prepared for treatments or analysis within the shortest possible time.

Preparation of Samples for Analysis

Tubers selected for analysis were first cut along the longitudinal axis, and only one-half of each tuber was diced and macerated in a Waring Blendor. Maceration was continued for five minutes after the broken tissues began to circulate. The resulting finely minced material was thought to represent an accurate blend or mean of tubers from a single plant. It was assumed that the variation in solanine content of such samples was less than would be obtained from a combination of tubers from several hills.

In order to prevent undesirable action of oxidative and hydrolytic enzymes, the pulverized potato tissue was weighed in duplicate 25-gram samples in small, tightly closed tin cans, and immediately quick-frozen and kept in deep-freeze storage at 0° F. To provide replacement material, where it might be required, part of the ground tissue was quick-frozen and stored in one-quarter pint waxed paper containers. At a convenient time, when analysis was to be carried out, the samples were quickly defrosted and prepared for extraction. Additional notes on maintenance of samples are presented in Appendix VI.

ANALYTICAL PROCEDURES(1) "Total solanine" determination

Early methods of solanine analysis in tuber and plant material were time-consuming and cumbersome, the many necessary steps often resulting in data so variable and inconsistent as to be of very doubtful value. Repeated modifications have been reported (41, 47, 59). In the extract, solanine may be estimated by gravimetric (5), hydrolytic (47) or colorimetric (41) methods. The most accurate and sensitive is the colorimetric estimation, which includes both solanine and solanidine if the free alkaloid is present. The resulting determination is most accurately designated as "total solanine." Unless otherwise stated or inferred, the term "solanine" is used herein as meaning total solanine.

Although no satisfactory extraction procedure for solanine analysis has been reported, a significant improvement in quantitative extraction procedure was introduced by Dabbs (15), and this method was used with slight modifications in earlier work by the present author (62). The same procedure has been used in the present investigation. Briefly, the method consists in the extraction of 25 gm. of macerated fresh tuber tissue (including the skin) with acidified ethylalcohol, using Soxhlet extraction apparatus and the Dabbs colorimetric method for total solanine estimation.

A Fisher AC model electrophotometer, using a B525 green filter (525 mμ), was used for colorimetric work, after the instrument was standardized using purified solanine. The calibration regression line was, for convenience, calculated on a 50 ml. volume basis. Total solanine concentrations, usually expressed in milligrams per 100 gm. of fresh or dry weight, are presented herein in parts per million (p.p.m.) of fresh tuber weight. The presented solanine data are all based on results of duplicate analyses.

(2) Solanine estimation by the hydrolytic method

The hydrolytic method, introduced by Conner (14) and improved by Rooke et al (47), estimates solanine by measuring the increase of reducing power on acid hydrolysis. The latter method uses the Hulme and Narain modification (26) of the ferricyanide method of reducing sugar determination (22). This method, which was used in the present work, estimates only solanine, and not solanidine.

An aliquot of ten ml. of solanine extract is pipetted into a graduated 30 ml. boiling tube fitted with a condenser (8-inch length of glass tubing in a rubber stopper). An equal volume of 5% hydrochloric acid is added, and the mixture is boiled on a water-bath for one hour. The solution is then cooled in tap water for 3-5 min., and neutralized immediately with 5 N sodium hydroxide (methyl orange), and made up to 25 ml. volume. A 10 ml. aliquot

of the neutralized solution is transferred into a wide 30 ml. test-tube, 5 ml. of alkaline potassium ferricyanide solution is added, and the mixture is again boiled on a water-bath under a condenser for 15 min. The test-tube is again cooled and additions are made of 5 ml. of potassium iodide reagent and 3 ml. of 5% acetic acid. The mixture is titrated with approximately N/75 sodium thiosulphate, using as an indicator a 1% starch solution saturated with NaCl, until the disappearance of the blue color.

A blank determination is made by using 10 ml. solanine extract neutralized immediately and titrated as above, after boiling the mixture with the alkaline ferricyanide solution. The difference between both values measures the increase of reducing power due to hydrolysis of solanine (equivalent of reduced ferricyanide).

The N/75 thiosulphate solution is standardized each day, using an acidified 2% potassium iodide solution and starch as indicator, and is calculated in terms of 0.01 normality.

The solanine equivalent of the unknown extract aliquot is then calculated with reference to a regression line constructed by the use of known solanine concentrations treated in the above manner. The regression line was again conveniently calculated per 50 ml. volume, the final volume in which the extracted solanine was dissolved prior to colorimetric or hydrolytic methods.

Figure 2. Calibration line for colorimetric (42) total solanine determinations, using purified solanine prepared in 10, 20, 30 and 40 p.p.m. concentrations.

Figure 3. Calibration line for hydrolytic (48) solanine determinations, using purified solanine prepared in 40, 60, 80, 100, 120, 140 and 160 p.p.m. concentrations.

The narrow range of thiosulphate volume required for the determination (0 to 3.0 ml.) necessitates the use of a precision burette graduated in 1/100 ml. The method is reliable only for solanine concentrations exceeding 150 p.p.m. fresh tuber material, with a reported accuracy of $\pm 5\%$ (47).

(3) Estimation of solanidine

The amount of solanidine present may be estimated only by the difference between colorimetric ("total solanine") and hydrolytic (solanine only) readings. A direct method for estimation of the alkaloid in plant material has yet to be developed, and the results of the above determination procedure are subject to an accumulation of experimental errors that may be involved in the colorimetric and hydrolytic analyses.

EXPERIMENT ISOIL TYPES, GEOGRAPHIC LOCATIONS AND VARIETAL DIFFERENCES AS
THEY AFFECT TUBER SOLANINE CONTENT

Total solanine analyses were carried out on mature tubers of fifteen potato varieties produced in 1953 on three representative soil types, as noted previously. The three locations were at Brooks, Fallis and Edmonton.

Despite the fact that some possibly important uncontrolled factors, such as fertility levels and variation in weather, were operative in the trial, new and useful information was anticipated. The solanine concentrations of Alberta-grown potatoes might deviate from those reported by Wolf and Duggar (59) who used tubers produced under very different environmental conditions. Moreover, there is no clear evidence that potatoes when removed from the soil are already bitter, except those that are sunburned, although this assumption is encountered frequently in reports on solanine research.

Experimental Material

It was recognized that tuber samples should be prepared for analysis with the shortest possible time lapse after harvest; and that during harvest all factors suspected of in-

1. Introduction

The purpose of this study is to investigate the effects of the proposed system on the performance of the system.

The study is organized as follows:

The first section describes the system and the proposed system.

The second section describes the experimental setup and the results of the experiments.

The third section discusses the conclusions and the future work.

The fourth section describes the organization of the paper.

The fifth section describes the system and the proposed system.

The sixth section describes the experimental setup and the results of the experiments.

The seventh section discusses the conclusions and the future work.

The eighth section describes the organization of the paper.

The ninth section describes the system and the proposed system.

The tenth section describes the experimental setup and the results of the experiments.

The eleventh section discusses the conclusions and the future work.

The twelfth section describes the organization of the paper.

The thirteenth section describes the system and the proposed system.

The fourteenth section describes the experimental setup and the results of the experiments.

The fifteenth section discusses the conclusions and the future work.

2. System Description

The system is designed to provide a secure and efficient way of managing the system.

The system is based on the following principles:

The system is designed to be secure and efficient.

fluencing increases in solanine should be controlled. This applies particularly to exposure of tubers to light and to low temperatures (62). This task was not achieved to complete satisfaction, as over 500 samples for all experiments had to be harvested, transported and prepared quickly for storage at 0° F. The principal deviation from the desired conditions occurred in the transport of tubers from the Brooks trial to Edmonton, during which very cold autumn weather was encountered. It was not possible to prevent some tuber exposure to low temperatures (35° F. range).

During the growing season the plants showed normal growth, producing somewhat smaller vine growth and tuber yield in the gray-wooded soil; however, satisfactory uniform samples of four to six tubers, each about 100 gm. in weight, were harvested from randomly chosen single hills. The conditions of harvest may be important and will be briefly discussed.

The experimental plot on the brown-soil zone at Brooks was harvested first on September 30, followed by harvest on October 5 at Fallis of the gray-wooded soil, and that on black soil on October 6 at Edmonton. Single-hill samples were collected in paper bags, one from each block (i.e., four per variety), designated with block (replicate) number, and placed immediately under a thick cover of jute sacking. The harvest at Edmonton was carried out under ideal warm and cloudy conditions, while a

bright sunny day prevailed during harvest at Fallis. The least desirable conditions were encountered during the harvest at Brooks, when rather low temperatures (about 40° F.) and a very bright sunny day were experienced. All samples after harvest were placed at 45° F. storage in complete darkness, and were processed as soon as possible for analysis. The task could not be completed in less than two weeks after harvest, and it is thought, as will be discussed later, that certain undesirable conditions during this period may have influenced solanine concentrations. The total number of variates for this experiment included three locations, four blocks (replicates) and 15 varieties; 60 samples from each location, and 12 for each variety, making a total of 180 samples.

Experimental Results and Discussion

A considerable variation of solanine concentrations was encountered in replicated samples from the same locality and within varieties on all three sites. Such a variability within a single variety - and even between tubers of a single hill - has been observed previously in potato bitterness investigations (27, 62). This characteristic makes the correct interpretation of the results the more difficult.

Site influence

Solanine concentrations of the 15 potato varieties at the three locations are presented in Table 1, and complete

Table 1. Site and variety effects on solanine concentrations of potato tubers. Data are means of four replicates as p.p.m. fresh weight

Variety	Site			Mean and S.D.		Variation range	
	Brooks	Edmonton	Fallis			Min.	Max.
C-16	25	17	37	26	$\pm$ 10	16	36
Canus	66	49	32	49	22	27	71
Carter's E.F.	65	42	57	55	16	39	71
E. Dewey	84	69	73	76	16	60	92
E. Ohio	86	57	68	70	19	51	89
I. Cobbler	19	22	23	21	3	18	24
Katahdin	23	45	41	36	15	21	51
Latowski	52	35	31	39	12	27	51
Manota	72	48	41	54	19	35	73
Netted Gem	70	58	48	59	17	42	76
50-3	19	24	38	27	12	15	39
50-7	51	21	18	30	19	11	49
177	18	21	17	19	12	7	31
Penner's Blue	24	26	45	32	11	21	43
Warba	35	39	55	43	14	29	57

The following table shows the results of the analysis of the data collected during the experiment. The data were collected from the experiment conducted on the 10th of May 1960. The data were collected from the experiment conducted on the 10th of May 1960.

No.	Analysis of variance						Total
	1	2	3	4	5	6	
1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1
21	1	1	1	1	1	1	1
22	1	1	1	1	1	1	1
23	1	1	1	1	1	1	1
24	1	1	1	1	1	1	1
25	1	1	1	1	1	1	1
26	1	1	1	1	1	1	1
27	1	1	1	1	1	1	1
28	1	1	1	1	1	1	1
29	1	1	1	1	1	1	1
30	1	1	1	1	1	1	1
31	1	1	1	1	1	1	1
32	1	1	1	1	1	1	1
33	1	1	1	1	1	1	1
34	1	1	1	1	1	1	1
35	1	1	1	1	1	1	1
36	1	1	1	1	1	1	1
37	1	1	1	1	1	1	1
38	1	1	1	1	1	1	1
39	1	1	1	1	1	1	1
40	1	1	1	1	1	1	1
41	1	1	1	1	1	1	1
42	1	1	1	1	1	1	1
43	1	1	1	1	1	1	1
44	1	1	1	1	1	1	1
45	1	1	1	1	1	1	1
46	1	1	1	1	1	1	1
47	1	1	1	1	1	1	1
48	1	1	1	1	1	1	1
49	1	1	1	1	1	1	1
50	1	1	1	1	1	1	1
51	1	1	1	1	1	1	1
52	1	1	1	1	1	1	1
53	1	1	1	1	1	1	1
54	1	1	1	1	1	1	1
55	1	1	1	1	1	1	1
56	1	1	1	1	1	1	1
57	1	1	1	1	1	1	1
58	1	1	1	1	1	1	1
59	1	1	1	1	1	1	1
60	1	1	1	1	1	1	1
61	1	1	1	1	1	1	1
62	1	1	1	1	1	1	1
63	1	1	1	1	1	1	1
64	1	1	1	1	1	1	1
65	1	1	1	1	1	1	1
66	1	1	1	1	1	1	1
67	1	1	1	1	1	1	1
68	1	1	1	1	1	1	1
69	1	1	1	1	1	1	1
70	1	1	1	1	1	1	1
71	1	1	1	1	1	1	1
72	1	1	1	1	1	1	1
73	1	1	1	1	1	1	1
74	1	1	1	1	1	1	1
75	1	1	1	1	1	1	1
76	1	1	1	1	1	1	1
77	1	1	1	1	1	1	1
78	1	1	1	1	1	1	1
79	1	1	1	1	1	1	1
80	1	1	1	1	1	1	1
81	1	1	1	1	1	1	1
82	1	1	1	1	1	1	1
83	1	1	1	1	1	1	1
84	1	1	1	1	1	1	1
85	1	1	1	1	1	1	1
86	1	1	1	1	1	1	1
87	1	1	1	1	1	1	1
88	1	1	1	1	1	1	1
89	1	1	1	1	1	1	1
90	1	1	1	1	1	1	1
91	1	1	1	1	1	1	1
92	1	1	1	1	1	1	1
93	1	1	1	1	1	1	1
94	1	1	1	1	1	1	1
95	1	1	1	1	1	1	1
96	1	1	1	1	1	1	1
97	1	1	1	1	1	1	1
98	1	1	1	1	1	1	1
99	1	1	1	1	1	1	1
100	1	1	1	1	1	1	1

data, including calculated data for analysis of variance, are presented in Appendix I.

Potato samples from the brown soil plots show slightly higher levels as eight of 15 varieties recorded the highest means, while from the gray-wooded-soil zone five varieties, and from the black-soil zone only two varieties recorded the highest mean values. The difference, however, is not consistent, as the Brooks samples also showed five of the lowest means. Therefore, it is not surprising that when the data were subjected to analysis of variance the influence of locations was found to be of insignificant nature (F value of 2.42 compared to 2.95 required at the five percent level). There is a significant interaction of "locations x varieties" (F value of 3.70 compared to 1.85 required at the one percent level), and this is attributed to the fact that the highest mean values of all varieties are not consistent with the location. There is a possibility, too, that the slightly higher levels at Brooks and Fallis locations could be a result of the conditions during transport.

The degree of variability in solanine concentrations is demonstrated by the fact that even in replications there was a significant F value of 3.91, compared to 3.93 required at the one percent level. The greatest variability was encountered in Canus variety, which shows values from 43 to 89 p.p.m. solanine in brown soil, 30 to 34 p.p.m. in gray-wooded soil, and 27 to

71 p.p.m. in the black-soil zone; and similar variability is shown by Manota (52 to 92, 40 to 56, and 27 to 55 p.p.m., respectively).

The foregoing considerations have led to the assumption that the differences in solanine concentrations are due rather to natural variation than to the influence of soils in different locations. Therefore, it seemed plausible to illustrate total solanine levels for each variety as means of replicates at all three plots. These data are presented in Table 2.

Table 2. Solanine concentrations in p.p.m. fresh tuber weight. Eight potato varieties grown at three locations. Data are means of 12 determinations.

Variety	Solanine p.p.m.		Variety	Solanine p.p.m.
Early Dewey	76		Katahdin	36 (55)
Early Ohio	70	(48)*	Penner's Blue	32
Netted Gem	59	(47)	50-7	30
Carter's E.F.	55		50-3	27
Manota	54		C-16	26
Canus	49		Irish Cobbler	21 (56)
Warba	43		177	19
Letowski	39			

* The readings in parentheses are normal concentrations found by Wolf and Duggar (59).

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The calculated deviation ranges of varieties showing maximum and minimum levels are illustrated in Figure 4. Standard deviations of 22 and 19 p.p.m. for Canus and for 50-7 indicate the extreme variation in solanine, which suggests an instability of varietal character as far as alkaloid synthesis is concerned. On the other hand, the extremely low variability of Irish Cobbler (3 p.p.m. deviation for 12 samples) denotes a high stability in this regard, which is noted to lesser extent in the C-16 and Latowski varieties.

Varietal influence

The differences between varieties exceeded the one percent level of significance (F value of 7.12 compared to the required 2.8 for high significance). The highest and lowest mean solanine concentrations were in Early Dewey (76 p.p.m.) and in variety 177 (19 p.p.m.). These differences demonstrate that the rate of solanine synthesis is determined through heritable characteristics of the varieties.

As the main interest in potato bitterness investigation is centered about the well-known ability of the Netted Gem variety to synthesize large amounts of solanine within a short time, the remaining 14 varieties are compared to this variety on the basis of the calculated L.S.D. of 22 p.p.m. for the one percent level of significance. A division into two groups may be made arbitrarily on the following bases: (1) the varieties which

 

Admission of New Members

 

Figure 4. Maximum and minimum solanine concentrations in tubers of fifteen Alberta-grown potato varieties.
 Solid black column - threshold concentration. Crossbar - mean of 12 samples per variety.

normally produce solanine concentrations above 37 p.p.m. of fresh tuber weight; and (2) varieties in which mean concentrations do not exceed this limit.

The authority for the development of this classification is supported by the results of analyses on pairs of tuber samples suspected of being bitter and received from farmers in various locations of the Province. Netted Gem variety (always represented in these paired samples) has shown very high concentrations, while the other variety of the sample seldom reached a dangerous level. The normal solanine levels for Netted Gem and Early Ohio were higher than those determined by Wolf and Duggar (59), while those of Katahdin, Warba and Irish Cobbler were lower.

In spite of the large variation between replicates and localities, the individual concentrations of all 180 samples were quite low, and much below a level which could indicate bitterness observable to taste. Only eight samples out of 180 (4.4 percent) exceeded the 90 p.p.m. level (five at Brooks, two at Fallis and one at Edmonton), with the highest readings being found in Early Ohio (102 p.p.m.), Manota (100 p.p.m.) and Early Dewey (97 p.p.m.). The low percentage of samples reaching the 90 p.p.m. concentration demonstrates that, under the conditions of this trial, bitter tubers are not produced during the plant and tuber development in the field. (Exceptions would be green, sunburned tubers.) High solanine build-up, then, may most frequently be

a result of post-harvest conditions. Similar observations were made in an earlier phase of potato bitterness work, when over 150 mature tuber samples of Netted Gem, Katahdin and Carter's Early Favorite, prepared for analysis and quick-frozen within a few hours after harvest, did not exceed 50 p.p.m. values (62).

There is, then, a strong implication that a study of factors causing potato bitterness should concentrate on conditions after harvest, particularly the handling of potatoes during harvest and in the first stages of the post-harvest rest period. There is still a possibility that certain responses to growing conditions may be delayed and become operative only later during the storage period.

EXPERIMENT II

SOLAR RADIATION EFFECTS UPON THE SOLANINE CONTENT
OF MATURE TUBERS

This experiment represented the major phase of the potato bitterness work, in which confirmation was sought concerning certain conditions thought to be responsible for the disorder. The trial was designed to study the variables suggested under "Study Objectives."

Experimental Material and Treatments

The growing conditions of the plots at Brooks, Edmonton and Fallis, from which experimental material was taken, are discussed in an earlier section. Potato samples of eight randomly selected varieties were exposed to solar radiation for periods of eight and 72 hours, and the storage duration and the locations represented additional variables. The eight-week storage period was included to test possible differences that might not be apparent at the end of the irradiation treatments.

A total of 48 samples from Experiment I were used as control for the exposed and unstored tubers, in order to avoid unnecessary duplication of analyses on the same tuber material. These samples were designated as controls at the time when samples

for Experiment I were taken. An additional set of 48 samples was needed to control the effects of the eight-week storage treatment.

In each locality, 12 single-hill samples per variety (three from each replicated block) were chosen at random. To these 12 samples (two replicates of two storage durations and three exposure treatments), the future treatments were randomly assigned at harvest time.

The total number of variables thus included eight varieties, three localities, two replicate samples and six experimental conditions; totalling 288 samples for the whole experiment.

The exposure treatments were as follows: (a) no exposure, control samples; (b) washed, mature tubers exposed continuously for eight hours to direct sunlight in the field; (c) continuous open-air exposure for three days and three nights.

The macerated tubers were quick-frozen for solanine determination immediately at the end of the exposure periods, and after an eight-week storage period. In the latter case, the loss of weight due to the storage conditions had to be recorded for correction of solanine concentrations. In both cases, the temperature and humidity fluctuations were recorded.

The eight-hour solar-radiation exposure was carried out on October 11, 1953 (8:30 a.m. - 4:30 p.m.), and the 72-hour exposure from October 10 (5:00 p.m.) until October 13 (5:00 p.m.). The shorter exposure was applied on a warm, sunny day, although it was hoped that temperatures would be lower than actually prevailed. The temperature for three and one-half hours was above 60° F. (with a maximum of 63° F.), for four hours between 50° and 60° F., and for a half-hour was below 50° (minimum of 48° F.). The first half of the exposure was bright and sunny, while during the latter half the light became somewhat hazy. The humidity during exposure was above 80% at the start, decreased to 40% at the end of the day, and varied between 60% and 40% in the afternoon.

During the 72-hour exposure, peaks of 63°, 62° and 61° F. were recorded during the daytime, and overnight minima were 48°, 35° and 34° F. The total exposure below 40° F. lasted for 20 hours, above 60° F. for 10 hours, while in the remaining 42 hours the temperature was fluctuating between 40° and 60° F. The humidity readings were mostly above 80% during the night-time, while daytime readings fell below 40% only for four hours on the last day of exposure (minimum of 35% R.H.). The temperature during the eight-week storage period fluctuated between 44° and 48° F.

A summary of temperature and humidity data is presented in Appendix II-D.

Experimental Results and Discussion

In all varieties, except Irish Cobbler, striking solanine concentration increases were observed as a result of both periods of exposure, and relatively little change took place during the subsequent storage period. Only "varieties" and "exposure treatments" were found responsible for changes in solanine levels beyond the one percent level of significance, while the growing conditions of the three locations and the storage conditions did not seem to influence the synthesis of solanine to an appreciable extent.

Solar radiation exposure effects

The tremendous effect on solanine synthesis of solar-radiation exposures, and particularly of the longer period, is evident from the data in Table 3, and from the maximum concentrations of individual samples reached in the varieties Netted Gem (396 p.p.m.), Early Dewey (350 p.p.m.) and Early Ohio (313 p.p.m.). The original data appear in Appendix II-A. The analysis of variance gave an exceedingly high value of significance (F value of 180.76, as compared to 18.0 required at the one percent level), and the required L.S.D. value was exceeded in both solar radiation exposures (see Appendix II-B).

During the eight-hour exposure, a rapid rate of solanine synthesis is initiated and this ranges from 14 to 53 p.p.m.

Table 3. Solar radiation and storage effects on solanine concentration of potato tubers. Data are means of six replicates as p.p.m. fresh weight.

Variety	Ex- posure hours	Storage duration						Diff. between means
		Nil			8 weeks			
		Mean	S.D.	Increase	Mean	S.D.	Increase	
C-16	0	26	± 2		24	± 5		-2
	8	64	16	38	62	25	38	-2
	72	128	26	102	140	29	116	12
E. Dewey	0	71	20		77	22		6
	8	117	19	46	132	34	55	15
	72	267	57	196	327	56	250	60
E. Ohio	0	70	17		75	23		5
	8	115	26	45	129	33	54	14
	72	232	43	162	254	59	179	22
I. Cobbler	0	21	7		26	5		5
	8	36	8	15	45	25	19	9
	72	56	23	35	60	16	34	4
Katahdin	0	37	9		42	15		5
	8	60	10	23	71	26	29	11
	72	141	23	104	191	60	149	50
Netted Gem	0	58	10		42	15		-16
	8	111	39	53	117	35	75	6
	72	281	66	223	355	86	313	74
50-3	0	29	15		16	2		-7
	8	43	12	14	50	11	34	7
	72	124	34	95	84	29	68	-40
177	0	20	6		14	3		-6
	8	51	18	31	55	18	41	4
	72	173	73	153	132	36	118	-41
Exposure means	0	41	± 24		39	± 27		
	8	74	± 37	33	83	± 43	44	11
	72	175	± 85	134	193	± 114	154	20

with a mean increase over control of 33 p.p.m. In the 72-hour exposure, a further increase was noted (mean increase over control, 134 p.p.m.). In relation to the length of exposure, this increase does not appear to be proportional, but seems rather to be limited by the capacity of a variety to synthesize solanine, and also by some internal limiting factor. This limiting factor becomes more pronounced as the exposure treatment progresses, and might be linked either to metabolic changes or to an accumulation of the products of synthesis. The apparent reduction in the rate of synthesis could not be accounted for by the lower temperatures during the latter part of the 72-hour exposure. It will be shown in a later trial (Experiment V) that low temperatures promote, rather than decrease, the efficiency of light exposure in solanine synthesis.

The increases in solanine concentration as a result of solar-radiation exposure are very consistent. In the 48 samples taken from the short-exposure treatment, only two samples failed to exceed the concentrations of comparable control samples from the same location, with differences of only 3 and 14 p.p.m., respectively. The experimental error of the analytical method is of the order ± 3 percent. In the 72-hour exposure, with the exception of Irish Cobbler variety, the solanine concentrations were 3.3 to 8.6 times higher than in the control samples; and the concentrations of the eight-hour exposure samples were lower

Figure 5. The influence of exposure to solar radiation and subsequent storage upon the solanine content of eight potato varieties.

than those of the 72-hour exposure in all but one of the 48 samples.

Another fact to be noted from Table 3 is the shift of deviation ranges towards higher values. The standard deviations ranged in control samples from 2 to 20 p.p.m.; in the 8-hour exposure from 8 to 39 p.p.m.; and in the longest exposure from 38 to 53 p.p.m. Generally, the higher the concentrations of solanine in tubers, the larger are the deviations that are encountered, which indicates a high degree of variability in the response between individual tuber samples. This effect is illustrated in the histogram of Figure 5. Because of the extreme values and deviations in the 72-hour exposure, the analysis of variance was not carried out on the original non-homogeneous solanine data, but instead these data were subjected to the logarithmic transformation. The analysis of the transformed variance data appears in Appendix II-B.

It could be assumed that with an increasing rate of solanine synthesis, the supply of some of the precursory building materials sooner or later might become limited or exhausted, resulting in an eventual decreased rate of synthesis. The lag period may not be coincident in all samples and might account for the variability of final solanine levels.

Varietal influence on solanine

The differences between the varieties exceeded the one percent level of significance (F value of 151.08, as compared

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to 2.75 required at the one percent level). The differences are obvious from inspection of data in Table 3, from which will be noted the lack of response of the Irish Cobbler variety to synthesize solanine under conditions wherein some other varieties produced extremely high concentrations. This presents clear evidence that the rate, and possibly the extent, of synthesis is to a certain degree a varietal characteristic.

According to the solanine levels produced, three different classes of varieties may be arbitrarily distinguished:

- (1) Varieties in which the increase after the 72-hour exposure was in excess of 150 p.p.m.: Netted Gem (mean increase 223 p.p.m.), Early Dewey (196 p.p.m.), Early Ohio (162 p.p.m.), and variety 177 (153 p.p.m.). In the first three varieties, the alleged toxic level of 200 p.p.m. was exceeded, and these should be classed as varieties that are predisposed to develop a dangerously high degree of bitterness.
- (2) Varieties with an obvious ability to develop solanine readily, but where the mean increase is less than 150 p.p.m., include: Katahdin (104 p.p.m. mean increase), C-16 (102 p.p.m.), and 50-3 (95 p.p.m.). In these varieties, the synthesis of solanine appears to proceed at a sluggish rate. The apparent inability to reach the toxic level is assumed to be limited by an internal factor - such as lack or un-

availability of a precursory compound, or inhibition of a particular enzyme which may be required for one of the steps of the biochemical reaction chain.

- (3) The variety Irish Cobbler represents a class in which abnormal synthesis of solanine apparently does not take place. This may be due either to the presence of a "balancing" mechanism or absence of suitable precursory building material. The negligible increase of 35 p.p.m. for this variety, in which 80 p.p.m. concentration was exceeded only in three of the 48 exposed samples, is further grounds for the thesis that the ability to synthesize abnormal amounts of solanine depends on inherited characteristics.

It appears that varieties in class (1), which easily become abnormally bitter, are provided with a generous supply of building materials and a complete synthesizing mechanism. Moreover, this mechanism seems easily set in motion under favorable conditions. In varieties of class (2), the imperfections of a labile synthesizing mechanism might account for a sluggish synthesis up to a certain level at which some limiting factor prevents further build-up.

Site influence

The growing conditions at the three locations did not exert any significant influence upon the rate of synthesis,

although some effects appear to be reflected on solanine levels of some varieties at the end of storage, as indicated by the significant interaction "locations x storage x treatments." This is attributed to somewhat higher concentrations in the stored samples from Brooks in the short exposure, and from Edmonton and Fallis samples only in the 72-hour exposure, while unstored samples from Brooks showed higher concentrations for both periods of solar-radiation exposure.

Storage influences

Although the variance due to a period of storage proved negligible (F value less than one), some minor differences were noted. In the varieties 177 and 50-3, there are slight increases at the shorter light exposure, but decreases are observed at the longer exposure. At the 8-hour exposure, there was, in general, little change in solanine levels with the exception of the Early Dewey and Early Ohio varieties, which had concentrations greater than 100 p.p.m. to begin with. In the stored tubers of the 72-hour exposure, an additional increase was registered in the Netted Gem and Early Dewey varieties, which have a high solanine production potential (74 and 60 p.p.m., respectively). Varieties 50-3 and 177, as mentioned above, actually decreased in solanine content in storage after the tubers had received the three-day exposure. These decreases were 41 and 40 p.p.m., respectively, and are thought to be caused by relative-

ly high weight losses (mean 3.3% in 50-3 and 4.4% in 177) which could have resulted from a tissue rupturing, for in many cases the latter two varieties showed a severe cracking of tubers within a short time after the harvest. Rupturing of cell tissues could induce activity of enzymes participating in hydrolysis and degradation of the solanidine molecule, although we have no experimental evidence to support this suggestion.

The slight solanine content increase in other varieties might be due to experimental error or the variability of solanine within the tuber samples, except in the case of Katahdin, where an additional synthesis and a mean increase of 50 p.p.m. was noted. This increase was found to be due to a relatively large difference in the solanine levels of unstored and stored 72-hour-exposure samples from the Edmonton site (127 and 252 p.p.m., respectively). In view of the sluggish rate of synthesis in all other samples of Katahdin, this increase seems to be paradoxical.

EXPERIMENT IIITHE INFLUENCE OF PHOTOPERIOD UPON POTATO TUBER SOLANINE CONTENT

An experimental plot of Netted Gem and Katahdin potatoes, grown at the University during 1953, was used for treatments designed to determine whether the photoperiod during the tuber-set season had an influence upon solanine concentration in mature tubers.

The shortening of the photoperiod was achieved by the use of a wooden frame covered with black cloth (Fig. 6), of 3 x 5 x 6 dimensions in feet. The frame could be easily set in place over 12 plants of two adjacent rows of Netted Gem and Katahdin varieties. The rows selected were on the north side of the plot, the next row to the south being planted four feet away, and a two-foot space was left between the frame and the next plants in the two treated rows. The duration of daylight available to the plants was reduced to eight hours and 30 minutes by removal of the frame at 8:30 a.m. and replacement of it at 5:00 p.m. This treatment was applied during the tuberization stage from July 20 until harvest on September 22, 1953, a total of 56 days. Adjacent plants unaffected by the shade of the box received the normal long photoperiod of 14 to 16 hours under Central Alberta conditions.

Figure 6. Netted Gem and Katahdin plants grown for Experiments III and IV. Note the shading frame used for shortening the photoperiod.

The plants under short and long photoperiods showed normal development, although an earlier maturity of vines and slightly smaller tubers and lower yields were observed in plants under the short photoperiod. Low yields, however, were common to all plants of the plot, probably due to relatively late planting (June 4) and to heavy rainfall and little sunshine during the season. The potatoes were harvested 110 days after planting.

Five to seven tubers of medium size were selected from single-hill samples, one-half of them being prepared immediately for analysis, the other half being first stored for an eight-week period. The loss of weight occurring in storage was recorded for correction of solanine concentrations.

The total number of variables included two varieties, two photoperiods, two storage durations and three replicates - a total of 24 samples.

Experimental Results and Discussion

The analytical results, presented in Table 4, show very low solanine concentrations in all samples, particularly in the Netted Gem variety.

There is a trend towards higher levels in tubers produced under shorter photoperiod in both varieties, but this effect may be the result of having to use somewhat smaller tubers for the short photoperiod samples. The sample weights are recorded

Table 4. The effect of short and long photoperiods upon solanine content of Netted Gem and Katahdin potatoes, at harvest time and after eight weeks in storage.
Data as p.p.m. of fresh weight.

Variety	Photoperiod	Replicate	Storage duration			
			N i l		8 weeks	
			Solanine p.p.m.	Mean	Solanine p.p.m.	Mean
Netted Gem	Long (control)	1	9		19	
		2	12	12	20	19
		3	16		17	
	Short	1	31		13	
		2	26	26	20	19
		3	21		25	
Katahdin	Long (control)	1	24		21	
		2	22	21	13	15
		3	16		12	
	Short	1	37		35	
		2	22	28	29	30
		3	26		25	

in Appendix III. Solanine levels in subsequent storage show insignificant differences from those extracted at harvest time.

Considering the differences of means, which differ at most by 14 p.p.m. in Netted Gem and 15 p.p.m. in Katahdin, the

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 UNDER THE DIRECTION OF PROFESSOR WILLIAM D. HAMILTON, AND
 WITH THE ASSISTANCE OF MR. J. H. HARRIS.

SOLUBLE		INSOLUBLE		MIXED		ANALYSIS
NO.	NAME	NO.	NAME	NO.	NAME	
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4	...	5	...	6	...	
7	...	8	...	9	...	
10	...	11	...	12	...	...
13	...	14	...	15	...	
16	...	17	...	18	...	
19	...	20	...	21	...	...
22	...	23	...	24	...	
25	...	26	...	27	...	
28	...	29	...	30	...	...
31	...	32	...	33	...	
34	...	35	...	36	...	
37	...	38	...	39	...	...
40	...	41	...	42	...	
43	...	44	...	45	...	
46	...	47	...	48	...	...
49	...	50	...	51	...	
52	...	53	...	54	...	

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variation appears to be due to natural solanine variability or to analytical procedure, rather than to the imposed conditions. Because of the very low concentrations found in all cases, no statistical analysis of the data was carried out.

Although minor differences between short and long-photoperiod tubers were noticed, the results can be considered as negative with respect to photoperiod effect, and as additional evidence that solanine concentrations of freshly harvested tubers are generally low and within normal variation, as concluded from the results of Experiment I. Tuber size is known to influence solanine concentrations (6, 59), and the variation found within this trial may well be due to that factor and to experimental error. Thus it means that a decrease of total radiant energy is not likely to be responsible for abnormal solanine synthesis. Moreover, the negligible changes observed after the eight-week storage period exclude the possibility that the photoperiod during tuber development may exercise a delayed effect during storage.

EXPERIMENT IV

LIGHT AND TEMPERATURE EFFECTS ON SOLANINE IN STORED TUBERS

Earlier work on this phase of the bitter-potato problem has suggested that illuminated tubers can show different degrees of bitterness depending upon prevailing temperatures (62). The trial reported here was undertaken to determine, if possible, how direct a relationship might exist between temperature and light from the standpoint of solanine build-up.

Experimental Material and Procedure

The material for the experiment was collected from the 1953 Edmonton plot wherein plants also were treated for photo-periodic effect. Tubers of Netted Gem and Katahdin varieties were utilized for the trial, which was carried out in two independent phases: (1) Tubers were placed in continuous dark storage at two temperatures (44° - 48° F. and 54° - 62° F.) for periods up to eight weeks; and (2) a continuous illumination of tubers in the first eight days after harvest. The tubers in (2) also were subjected to the same two temperature levels. Samples in triplicate for each treatment were analyzed for total solanine at intervals of 0 (control), 2, 4, 6 and 8 weeks or days, respectively. Illumination was provided by a 100 W. Mazda lamp suspended about 30 inches above the exposed tubers.

Control samples were prepared for analysis and stored in the frozen state on the day of harvest (October 16). The samples stored in darkness were kept from October 16 until December 11, and the tubers necessary for a three-replicate sample were removed for analysis at two-week intervals.

The tubers placed in the lighted storage were sampled at two-day intervals between October 22 and 30. Humidity and temperature data for both phases of the trial were recorded, but the fluctuations were too slight to have had any influence upon the main factors being tested. Sample weights were recorded at the start and the finish of each treatment, and the solanine concentrations were corrected for weight losses where necessary.

The number of variables included two temperatures, two varieties, five sampling dates and three replicates, giving a total of 60 samples for each of the two divisions of the work.

The desirability of weekly sampling in the case of dark vs. light stored tubers, and daily sampling in the effect of continuous illumination trial, was suggested by preliminary observations, in which solanine increases occurred slowly in tubers kept in the dark, and near-toxic levels were reached within a week after illumination (62). Because of the differences in sampling dates, both trials have to be discussed and tested for significance independently.

Experimental Results and Discussion

- (1) The effect of temperature on the rate of solanine synthesis in darkness.

The mean solanine concentrations are presented in Table 5, and the complete data, including storage weight losses, are given in Appendix IV.

Table 5. Solanine concentrations of potato tubers stored at two temperatures in the absence of light. Data are means of three replicates, p.p.m. fresh weight.

Variety	Temperature	Storage duration (weeks)					Regression $y = a + bx$	Correlation coefficient
		0	2	4	6	8		
Katahdin	Low	25	43	40	36	39	$32.7 + 1.0 x$	+0.261
	High	25	45	61	50	62	$32.9 + 3.9 x$	+0.746 **
Netted Gem	Low	28	33	40	68	76	$22.5 + 6.6 x$	+0.781 **
	High	28	50	53	67	85	$30.1 + 6.6 x$	+0.791 **

Note - y = solanine concentration; x = storage duration;
required "r" value at the 1% level for 14 D.F. = 0.641.

The results presented here indicate that two varieties do show an increase of solanine levels that varies positively with the temperature of the storage. Netted Gem tubers show

a slow increase in glycoalkaloid content at both temperatures, while Katahdin does so only at higher temperatures. The differences are statistically significant, as illustrated by the regression lines in Figure 7 and Table 5.

There is some doubt as to whether a toxic level of 200 p.p.m. fresh tuber weight would be reached in a normal storage period, in view of the slow rate at which the glycoalkaloid was synthesized under these conditions. The maximum solanine content recorded for any sample was 104 p.p.m. in one Netted Gem lot after the full eight weeks' storage. The largest overall increase is seen in the first two-week interval of storage, but in Netted Gem at the low temperature the rise appears steady and nearly proportional, indicating the absence of any interfering factor or factors. The relative irregularity of the rate of increase at the high temperature probably is due to differences in the metabolic state or the degree of maturity of the sample tubers; an effect, in any case, that must be caused by the higher temperature.

The analysis of variance (see Appendix IV-B) shows no significant difference between the two varieties, but a significant difference exists in the interaction of "variety x storage duration" ($F = 4.3$, compared to $F = 3.86$ required at the one percent level). This is undoubtedly a result of the inconsistent response of Katahdin tubers at low temperature at the six and eight-week exposures. The lower values for Katahdin

Figure 7. Changes in solanine concentrations of Netted Gem and Katahdin potatoes stored at two temperatures under continuous darkness.

samples at low temperature for the last two sampling periods indicate differences in the rate of glycoalkaloid synthesis.

The steady rate of solanine increase at low temperature in Netted Gem tubers appears to be due to an uninhibited metabolic process. The respiratory rate which presumably is low under these conditions seems to favor the formation of secondary nitrogen products. In Katahdin at low temperature there is apparently a limiting factor which is removed or inactivated at high temperatures, and which may eventually be directly linked with respiration. A better understanding of the fluctuations might be feasible if changes in both free alkaloid and the glycoside could be recorded - an extension of the study which was not possible here. Moreover, there is presently no reliable solanine test for tubers having a relatively low total solanine content.

(2) Light and temperature effects on the rate of solanine synthesis

The analytical data in Table 6 show a consistent increase of mean solanine concentrations in all four conditions tested, although marked differences are evident in the behaviour of the two varieties. The difference between varieties is significant (F value of 9.95, compared to 7.35 required for the one percent level). The effect of temperature is insignificant and

Table 6. Solanine concentrations of illuminated potato tubers. Determinations made at two-day intervals, and as p.p.m. fresh weight in three replications.

Variety	Temperature	Storage duration (days)					Regression $y = a + bx$	Correlation coefficient
		0	2	4	6	8		
Katahdin	Low	25	35	42	54	70	$25.7 + 0.49x$	+0.740 **
	High	25	95	99	100	126	$49.0 + 10.3x$	+0.795 **
Netted Gem	Low	28	90	122	144	170	$42.9 + 17.0x$	+0.918 **
	High	28	69	92	116	118	$39.0 + 11.4x$	+0.863 **

Note - y = solanine concentration; x = storage duration;
required "r" value at the 1% level for 14 D.F. = 0.641.

the "temperature x variety" interaction is significant, as anticipated by noting the more rapid solanine increase in Katahdin at high temperatures and in Netted Gem at the lower temperatures.

All four correlation coefficients were found to be significant, which can be clearly noted from the slope of regression lines in Figure 8.

The glycoalkaloid increase is quite rapid during the early part of illumination, except in the case of Katahdin tubers stored at low temperatures. In the first two days, four samples out of 12 exceeded the 90 p.p.m. value, and by the eighth day nearly one-half of all determinations were higher than this

Figure 8. Changes in solanine concentrations of Netted Gem and Katahdin potatoes stored at two temperatures under continuous illumination.

figure. The maximum reading for Netted Gem was 191 p.p.m. (low temperature), and for Katahdin 141 p.p.m. (high temperature). The rate of increase in Netted Gem tubers at high temperature was sharply reduced after the initial quick increase, but at high temperature the rise was steady and suggests no interference of any limiting factors.

The steep regression lines in Fig. 8 show that if the illumination period is continued long enough, ultimately a sufficient amount of solanine will be produced to exceed the toxicity level of 200 p.p.m. This is particularly true with Katahdin tubers at the higher temperature, and with Netted Gem tubers under cooler conditions, a paradoxical result that suggests different genetic constitutions with respect to some inhibiting or activating factor or factors. It is obvious that at any normal temperature range the effect of light exposure on tubers is to induce solanine synthesis. Varietal differences in content of solanine, and in the rate of solanine increase, are significant variables. Also, the interactions "variety x storage duration" and "variety x temperature" are significant, a result that is consistent with the data recorded in Table 6.

The steady rise of solanine levels (in Netted Gem) appears to indicate an activation of a "solanine-balancing" factor, and such a factor may not be present in the variety Katahdin. On the other hand, the differences observed might be

due to varietal differences in pathways of metabolism and this may not be linked directly to respiration.

It is interesting to note that the loss of tuber weight during the storage in absence of light is considerably higher than in the case of illuminated tubers, particularly towards the end of the storage (see Appendix IV-C). Thus it appears that illumination results in a decreased respiratory rate (less weight loss), which may consequently favor secondary metabolic processes at the prevailing higher temperatures. With the exception of Netted Gem potatoes illuminated at low temperatures, an increased rate of solanine synthesis was noted at the higher temperatures.

EXPERIMENT VSOLANINE AND SOLANIDINE IN BITTER NETTED GEM POTATOES

The relationship of the glycoside and free alkaloid in potatoes has received little attention. Solanidine is known to occur in sprouts of some potato varieties (13), and is also reported to appear in brown senescent potato vines (37). Another view is that the alkaloid does not occur naturally in free state and that its appearance might be due to the activity of certain hydrolyzing enzymes during the preparation of samples for solanine analysis (50). Accurate studies on solanidine are difficult because at the present time the only practical method of estimation in plant material is based on the difference between colorimetric and hydrolytic methods of solanine analysis, the latter of which requires a minimal total solanine amount of 150 p.p.m. of fresh sample weight (47). The presence of solanidine, therefore, cannot be determined in tubers with a low or normal content of solanine. During the 1954-55 season, potato samples of a highly bitter nature were very frequently secured for analysis, so obtaining suitable material for solanidine determinations was a simple matter. Also, highly bitter tuber material could be developed very readily by using solar radiation as an induction technique. The unusually high amounts of solanine that occurred frequently in the 1954 Alberta potato crop led to the question

as to whether this might be due to an accumulation of solanidine. The synthesis of the alkaloid undoubtedly precedes that of solanine. The following report outlines the procedure and results of an effort to determine whether solanidine is actually present in the free state in bitter tubers, and, if so, to establish its distribution within the tubers.

Experimental Material

Samples of tubers in triplicate were exposed late in November, 1954, to solar radiation for periods of three, five and seven days. For most of the exposure period, the weather was bright, sunny and cool, with temperatures ranging from 26° to 59° F., but remaining most of the time below 50° F. Light frost was recorded during three overnight periods, with minima of 26°, 32° and 28° F. on the first, fourth and sixth nights. The tubers were prepared immediately for analysis at the conclusion of each exposure period, and solanine determinations by both colorimetric and hydrolytic methods were carried out. For the latter procedure, the method described by Rooke et al (47) was used. These methods have been described in a special section of this thesis.

Additional material was available from a number of bitter potato samples received from various parts of the Province. Solanine and solanidine determinations were carried out, and in

 

President of the United States

 

a sufficiently large sample received from Belmont, Alberta, separate determinations were made on peel and tuber flesh in order to determine the amount of solanine removed by peeling and the distribution of the glycoalkaloid, and of solanidine if present.

Experimental Results and Discussion

The results of solanine analyses by both methods on artificially embittered tubers are presented in Table 7. The

Table 7. The effect of autumn exposure to solar light upon solanine and solanidine in Netted Gem potatoes.
Data are as p.p.m. of fresh weight.

Sample	Exposure days	Methods of solanine analysis		Difference (solanidine)
		Colorimetric	Hydrolytic	
1	3	510 *	347 *	163
2		645	382	263
3		525	352	173
1	5	515	352	163
2		619	347	272
3		530	380	150
1	7	697	386	311
2		837	411	396
3		863	355	508

* Each reading is the mean of duplicate determinations.

data show rather wide differences, so that the presence of the free alkaloid is strongly indicated. It is unlikely that such consistent differences could be due to the lesser accuracy of the hydrolytic method.

The results of the first phase of this trial confirm the view that temperatures near freezing point in combination with solar radiation are highly efficient in producing extreme concentrations of total solanine.* It is interesting to note that the solanine as determined by the hydrolytic method shows a narrow range of variation from 347 to 441 p.p.m., while the amounts of solanidine range widely from 150 to 508 p.p.m. Thus it appears that within the short time in which a rapid rate of glycoalkaloid synthesis was induced the formation of the glycoside is limited to a certain degree by an internal factor.

The presence in the tubers of free solanidine, in amounts up to 50 percent or more of the total bitter components, indicates that the primary effect of solar radiation and low temperatures is the synthesis of this substance. It appears that precursory compounds for such synthesis must be present to a degree that permits, within limits, a steady increase in the rate of the glycoalkaloid build-up. The uniform levels of solanine point towards some intervening factor, such as a limited supply of the three sugars or an inhibition of the participating enzyme system.

* Initial levels of solanine ranged from 42 to 67 p.p.m.

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That the free solanidine is converted rather slowly to solanine is indicated by analyses of bitter potato samples, all of them of Netted Gem variety, which were received from a number of growers and consumers. These bitter tubers were received and prepared for analysis at the dates indicated in Table 8. It was assumed that the synthesis of solanidine occurred during or shortly after harvest, and considering the lapse of time, and the results shown in Table 7, it appears that the free alkaloid is eventually quantitatively converted at a slower rate to the glycoside. The significance of this process cannot be explained

Table 8. Solanine and solanidine analyses on potato tubers from Central Alberta sources, 1954 crop.

Date Received	Locality	Solanine, p.p.m.		Difference (solanidine p.p.m.)	Percentage	
		Colorimetric	Hydrolytic		Solanine	Solanidine
Dec.20/54	Edmonton(1)*	562	446	116	79.4	20.6
Jan.8/55	Edmonton(2)	726	581	145	80.0	20.0
Jan.21/55	Calmar	348	301	47	86.5	13.5
Jan.23/55	Red Deer	400	369	31	92.2	7.8
Jan.25/55	Strathmore	406	375	31	92.4	7.6
Jan.25/55	Belmont(1)**	560	486	74	86.8	13.2
Feb.5/55	Islay	515	485	30	94.2	5.8
Feb.20/55	Belmont (2)	571	543	28	95.1	4.9

* Edmonton (1), (2) - unrelated samples.

** Belmont (1), (2) - identical samples, analyzed at different dates.

without further collateral studies of internal metabolic changes, although from the scarce occurrence of solanidine in nature it might be postulated that relatively high concentrations may have a toxic effect upon the plant tissue itself and the conversion to the glycoside is only a natural defence mechanism within potato plants.

A study of the data presented in Table 8 indicates that tubers must possess some means for a conversion of the free alkaloid to the glycosidal form.

Once positive evidence had been secured of the presence of solanidine in bitter potatoes, an attempt was made to determine the relative distribution of both components of bitterness. At the same time, information was sought as to the amount of solanine removed by peeling of potato tubers. A 20-lb. sample of the bitter Belmont Netted Gem tubers was used for this investigation.

The analytical results are presented in Table 9, and illustrated in Figures 9, 10.

The peel represents only 13.9 percent of the total tuber weights; yet the peel and flesh contain the same amount of solanidine. Thus the concentration of solanidine in the peel is about six times higher than that in the tuber flesh.

The distribution of solanine and solanidine within a tuber is of practical significance in estimating the amounts

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Figure 9. Concentrations of solanine and solanidine in peels and tuber flesh of bitter Netted Gem potatoes.

Figure 10. Percentage distribution of solanine and solanidine in peels and tuber flesh of bitter Netted Gem potatoes.

Table 9. Percentage distribution and concentration of solanine and solanidine in peels and tuber flesh of bitter potatoes.
Data as p.p.m. fresh weight.

	Fresh wt.	Solanine		Solanidine		Total Solanine	
	%	p.p.m.	%	p.p.m.	%	p.p.m.	%
Percentage distribution							
- Peels	13.9	140	24.6	47	8.3	187	32.8
- Flesh	86.1	336	59.0	47	8.2	383	67.2
- Whole tuber*	100	476	83.6	94	16.5	570	100
Concentration							
- Peels	100	1006		338		1344	
- Flesh	100	391		54		445	
- Whole tuber*	100	476		94		570	

* Calculated.

which can be removed from bitter potatoes by peeling. The large amounts of solanine in the flesh of bitter potatoes seems most probably to be a result of diffusion from the site of synthesis, as it is difficult to visualize that the actual build-up could take place in the inner cortex or pith regions of the tubers. The extremely high concentration of "total solanine" in the peel (1344 p.p.m. fresh weight) lends support to the general supposition that synthesis is confined to this region.

Slight greening was observed in tubers exposed to sunlight (Table 7). Because of this, it appears that at least a partial synthesis of chlorophyll takes place in the earliest phase of exposure effect, and this is coincident to or followed by solanidine synthesis. It is highly probable that the photosynthetic rate exceeds that of respiration during the exposure and thus the energy products would be available for secondary metabolic conversion.

GENERAL CONCLUSIONS

A series of solanine determinations under broad environmental conditions and involving many varieties has established the fact that the poisonous glycoalkaloid does not occur in abnormal amounts in maturing potato tubers prior to harvest. This is counter to the conclusions of many earlier workers, but is supported to a degree by Pallman and Schindler (39) and Wolf and Duggar (59), who have shown that solanine levels in potato tubers decrease towards the end of the growing season.

It appears that the potato plant possesses some mechanism to control either the rate of solanine development within the tuber, or the translocation of the substance from stems and leaves into the tuber.

Solanine is maintained in the tubers at levels that at harvest are variable with the variety but that in no instance are high enough to cause bitterness, and it is postulated that a metabolic mechanism or a system of enzymes exert a control over the movement of solanine during normal growth. This does not exclude the possibility that environmental factors exert a delayed-action effect that may not be evident until several weeks or months after harvest.

Wolf and Duggar (59) have suggested that the low solanine content in tubers at maturity may be due to a "diluting"

effect of tuber enlargement. Whatever the reason, the experimental evidence presented here allows the conclusion that neither photoperiod alteration, soil type, nor geographic location (within Alberta) has an appreciable influence upon the glycoalkaloid content of the potato tuber. Earlier work at Edmonton has indicated that other environmental artifacts such as depth of planting, degree of hilling, top-killing, time of planting and application of fertilizers have no apparent influence upon whether or not bitterness develops in potato tubers (27, 62).

Once the tuber is removed from the soil, it is subject to an interaction of inherited and environmental factors, and under certain conditions the tuber may have initiated a rapid rate of solanine synthesis that in predisposed varieties leads to abnormal concentrations. The present work indicates that not only are the "normal" concentrations of mature tubers dominated by heritable characters, but also the potential ability of a variety to maintain normal levels of solanine appears to be inherited. Thus it was not possible to develop bitterness in Irish Cobbler tubers under the same conditions that led to extreme bitterness in Netted Gem tubers. It is well-known that in tobacco breeding strains have been produced differing in ability to synthesize nicotine (18, 19). Also, a strain of tomatoes (T414) has been shown recently to produce a bitter tasting fruit, suspected to be due to formation of a glycoalkaloid, and crosses of this strain in later progeny tests show varying

degrees of bitterness, indicating that the ability to produce the bitter principle is heritable (9). There is little doubt that the unidentified bitter component is similar, if not identical, with the glycoalkaloid tomatine, which has been reported as present in tomato plant leaves (43, 50). From these examples, and in view of the varietal differences observed during the present investigations, it appears certain that increased or reduced ability for solanine synthesis depends on inherited genetic factors.

Exposure of tubers to solar radiation showed that with most varieties a higher rate of solanine synthesis was evident when low temperatures were a coincident factor. Alternating light and dark periods (day and night) with a parallel influence upon metabolic processes within the tuber, may play a part in the solanine synthesis rate increase. It is thought that the availability of building materials may be related to the activity of enzymatic systems responsible for supplying and transforming compounds utilized in the individual steps of the alkaloid synthesis.

Tubers of different varieties that showed a close similarity in solanine content at harvest time were exposed to incandescent light and at the same time to low and high temperature storage. At the termination of the storage period, the variation in solanine levels was marked, indicating differential

varietal response to the conditions imposed. Varieties could be classified into three divisions: (a) those not subject to high concentrations under any conditions; (b) those capable of marked increase but not to levels of human toxicity (200 p.p.m.); and (c) those that readily reach dangerously high levels when environmental conditions are such as to encourage this synthesis. Positive effects of light, temperature and the interaction of the two factors also were observed. The variability encountered may be accounted for by differences in the stage of tuber rest period (metabolic state), tuber maturity and respiratory rate.

The influence of the light and low temperature factors is certain to be the principal cause of the abnormally severe bitterness in many Alberta 1954 grown potatoes. Dangerous levels of solanine were noted, particularly from Netted Gem tubers, and in most cases a history was available that included tuber exposure to light after harvest. The exposures varied from direct sunlight for a few hours to several days in subdued light (as on a garage floor), and were employed to dry off muddy tubers harvested from the wet, heavy soil. The 1954 Alberta potato harvest coincided with a period of bright but cool weather, following very wet growing conditions.

Potatoes that were not exposed to light prior to or during storage also showed a variety difference in the rate of synthesis of the poisonous compound during a two-month period.

Thus Katahdin tubers showed essentially no change at a low temperature but a moderate increase at the higher temperature. The reverse was true for tubers of Netted Gem variety, and the conclusion of many earlier workers that solanine could not be synthesized in dark storage has been proved incorrect. While it appears that for an increased rate of solanine synthesis in Katahdin tubers, a decrease in the respiratory rate (excluding the photochemical influence), and the consequent release of one or more specific metabolites, is essential, the rate of synthesis in Netted Gem tubers does not seem to be limited by such a factor.

The presence of free solanidine in bitter potatoes has now been established, and may point towards two separate phases or mechanisms of solanine production: (a) formation of the aglycone which proceeds rapidly at low temperatures in the presence of a photochemical stimulus; and (b) conversion to the glycoside, which does not necessarily require the presence of light. In all probability, the rate of the alkaloid conversion depends upon the availability of the three sugar components. Both processes appear to be controlled by genetic characteristics, since certain varieties only are able to produce high solanine concentrations. The formation of glycosides in at least two instances has been shown to be under the control of specific genes (3, 4); moreover, the necessity of photochemical reactions for the production of heterocyclic ring structures has been suggested by several authors (cit. 18).

At the present time, the study of glycoalkaloid or alkaloid formation in vivo is confined to heritable or extrinsic factors responsible for lower or higher rates of synthesis. The approaches of the organic chemist and botanist to the general subject reveal little information with regard to the involved biosyntheses. The lack of a unified approach is ascribed to three deficiencies of alkaloid biochemistry (18): (a) The absence of any evidence that would relate alkaloids to definite biochemical or biophysical functions and thus explain their significance in the metabolism of plants that produce them; (b) the inability of research workers to date to demonstrate a direct relationship of alkaloids to anabolic and/or catabolic processes in the plant; although several reports maintain that alkaloid formation is parallel or related to some phase of nitrogen metabolism (29); and (c) the powerful physiological effects of alkaloids introduced into the animal body are almost certainly concerned with some type of interference or inactivation of enzyme systems, while the presence or absence of alkaloids in the plant tissues always seemed a matter of relative indifference to the plant itself (18); which probably accounts for the fact that these substances are generally assumed to be irreversibly formed end-products of secondary metabolic activities (7).

The presence of solanine in tissues of high metabolic activity (33) and its degradation in senescent leaves (37), together with similar observations on Belladonna alkaloids (29),

point towards some necessary function of these compounds, although none of the present experimental techniques has been able to reveal such a function.

Unfortunately, until definite uses of biochemical or biophysical nature can be established, perhaps by determining the locus of synthesis and the sequence and intermediates of the biochemical reactions (as well as related physical or chemical processes), the comprehensive process of alkaloid biosynthesis will remain beyond understanding.

SUMMARY

The experimental evidence presented in this work established the following facts concerning initiation and the rate of solanine formation in potato tubers:

1. Solanine concentrations in mature tubers is generally low due to the very slow rate of solanine synthesis in tubers grown in the absence of light and/or to the existence of a low rate of translocation during the vegetative growth.
2. Abnormal rates of synthesis occur only in certain varieties possessing an inherited predisposition

for rapid solanine build-up if tubers of these varieties are exposed to favorable extrinsic conditions.

3. Normal solanine formation (content) appears to be a varietal characteristic, presumably heritable, but seldom accountable for toxic levels in bitter potatoes.
4. Abnormal synthesis of solanine requires a photochemical stimulus (solar radiation or artificial illumination), the efficiency of which appears generally to be increased by a decrease in the temperature at which the tubers are held. It is suggested that a "solanine synthesizing" mechanism is set in motion, or a "balancing" mechanism is inhibited by the effect of light and low temperatures.
5. A natural slight increase of solanine levels appears to take place in the early phase of the post-harvest rest period of the tuber, which may continue in some varieties for an extended period, while in others it may become limited by some unknown internal factor.
6. The aglycone solanidine is found in bitter tubers of Netted Gem variety, and under favorable conditions it is formed first at a very rapid rate, while the conversion to the glycoside appears to proceed at a slow and uniform rate.

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6. The sixth part is devoted to a study of the various conclusions of the investigation.	95
7. The seventh part is devoted to a study of the various suggestions for further work.	115
8. The eighth part is devoted to a study of the various references.	135
9. The ninth part is devoted to a study of the various indices.	155
10. The tenth part is devoted to a study of the various appendices.	175
11. The eleventh part is devoted to a study of the various tables.	195
12. The twelfth part is devoted to a study of the various figures.	215
13. The thirteenth part is devoted to a study of the various maps.	235
14. The fourteenth part is devoted to a study of the various photographs.	255
15. The fifteenth part is devoted to a study of the various diagrams.	275
16. The sixteenth part is devoted to a study of the various charts.	295
17. The seventeenth part is devoted to a study of the various graphs.	315
18. The eighteenth part is devoted to a study of the various curves.	335
19. The nineteenth part is devoted to a study of the various surfaces.	355
20. The twentieth part is devoted to a study of the various volumes.	375
21. The twenty-first part is devoted to a study of the various areas.	395
22. The twenty-second part is devoted to a study of the various lengths.	415
23. The twenty-third part is devoted to a study of the various widths.	435
24. The twenty-fourth part is devoted to a study of the various heights.	455
25. The twenty-fifth part is devoted to a study of the various depths.	475
26. The twenty-sixth part is devoted to a study of the various thicknesses.	495
27. The twenty-seventh part is devoted to a study of the various diameters.	515
28. The twenty-eighth part is devoted to a study of the various radii.	535
29. The twenty-ninth part is devoted to a study of the various angles.	555
30. The thirtieth part is devoted to a study of the various distances.	575
31. The thirty-first part is devoted to a study of the various times.	595
32. The thirty-second part is devoted to a study of the various speeds.	615
33. The thirty-third part is devoted to a study of the various accelerations.	635
34. The thirty-fourth part is devoted to a study of the various forces.	655
35. The thirty-fifth part is devoted to a study of the various pressures.	675
36. The thirty-sixth part is devoted to a study of the various temperatures.	695
37. The thirty-seventh part is devoted to a study of the various densities.	715
38. The thirty-eighth part is devoted to a study of the various masses.	735
39. The thirty-ninth part is devoted to a study of the various volumes.	755
40. The fortieth part is devoted to a study of the various areas.	775
41. The forty-first part is devoted to a study of the various lengths.	795
42. The forty-second part is devoted to a study of the various widths.	815
43. The forty-third part is devoted to a study of the various heights.	835
44. The forty-fourth part is devoted to a study of the various depths.	855
45. The forty-fifth part is devoted to a study of the various thicknesses.	875
46. The forty-sixth part is devoted to a study of the various diameters.	895
47. The forty-seventh part is devoted to a study of the various radii.	915
48. The forty-eighth part is devoted to a study of the various angles.	935
49. The forty-ninth part is devoted to a study of the various distances.	955
50. The fiftieth part is devoted to a study of the various times.	975

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1	1. The first part of the report deals with the general situation of the country and the progress of the work during the year.
2	2. The second part of the report deals with the results of the work done during the year.
3	3. The third part of the report deals with the financial statement of the year.
4	4. The fourth part of the report deals with the general remarks of the committee.
5	5. The fifth part of the report deals with the conclusions of the committee.
6	6. The sixth part of the report deals with the recommendations of the committee.
7	7. The seventh part of the report deals with the general remarks of the committee.
8	8. The eighth part of the report deals with the conclusions of the committee.
9	9. The ninth part of the report deals with the recommendations of the committee.
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18	18. The eighteenth part of the report deals with the recommendations of the committee.
19	19. The nineteenth part of the report deals with the general remarks of the committee.
20	20. The twentieth part of the report deals with the conclusions of the committee.

APPENDIX I

(DATA ON EXPERIMENT I)

A. Original data on solanine concentrations as p.p.m. fresh tuber weight. Fifteen potato varieties grown at three localities in Alberta.

1. BROOKS (Brown-soil zone)

Variety	Replicate				Mean and S.D.			Variation range	
	1	2	3	4				Min.	Max.
C-16	21	16	35	26	25	±	8	17	33
Canus	38	75	58	92	66		23	43	89
Carter's E.F.	50	72	84	52	65		14	51	79
E. Dewey	79	78	97	82	84		18	66	102
E. Ohio	97	82	63	102	86		18	68	104
I. Cobbler	13	13	26	24	19		7	12	26
Katahdin	22	18	21	30	23		2	21	25
Latowski	46	60	51	49	52		6	46	58
Manota	72	50	100	58	72		20	52	92
Netted Gem	89	83	50	59	70		19	51	89
50-3	24	12	22	16	19		2	17	21
50-7	47	28	52	75	51		19	32	70
177	14	22	12	25	18		2	16	20
Penner's Blue	26	23	15	32	24		2	22	26
Warba	41	29	33	35	35		5	30	40
Mean					47	±	27	20	74

(Appendix I-A - cont'd)

2. EDMONTON (Black-soil zone)

Variety	Replicate				Mean and S.D.			Variation range	
	1	2	3	4				Min.	Max.
G-16	19	19	14	14	17	±	3	14	20
Canus	51	38	78	28	49		22	27	71
Carter's E.F.	50	25	37	57	42		14	28	56
E. Dewey	57	46	78	96	69		22	47	91
E. Ohio	51	46	63	66	57		3	54	60
I. Cobbler	16	27	14	32	22		3	19	25
Katahdin	32	39	61	47	45		12	33	57
Latowski	31	19	41	48	35		12	23	47
Manota	39	55	43	54	48		8	40	56
Netted Gem	43	48	67	72	58		14	44	72
50-3	16	29	23	27	24		2	22	26
50-7	18	17	20	27	21		1	20	22
177	17	17	20	28	21		5	16	26
Penner's Blue	24	16	27	38	26		3	23	29
Warba	29	41	50	35	39		2	37	41
Mean					38	±	19	19	57

(Continued on page 102)

(continued from page 100)

Date		Time		Temperature (°C)				Remarks
Month	Day	Hour	Minute	Air	Soil	Water	Shade	
Jan	10	8	15	12	10	15	10	Clear
Jan	11	8	15	11	9	14	9	Clear
Jan	12	8	15	10	8	13	8	Clear
Jan	13	8	15	9	7	12	7	Clear
Jan	14	8	15	8	6	11	6	Clear
Jan	15	8	15	7	5	10	5	Clear
Jan	16	8	15	6	4	9	4	Clear
Jan	17	8	15	5	3	8	3	Clear
Jan	18	8	15	4	2	7	2	Clear
Jan	19	8	15	3	1	6	1	Clear
Jan	20	8	15	2	0	5	0	Clear
Jan	21	8	15	1	-1	4	-1	Clear
Jan	22	8	15	0	-2	3	-2	Clear
Jan	23	8	15	-1	-3	2	-3	Clear
Jan	24	8	15	-2	-4	1	-4	Clear
Jan	25	8	15	-3	-5	0	-5	Clear
Jan	26	8	15	-4	-6	-1	-6	Clear
Jan	27	8	15	-5	-7	-2	-7	Clear
Jan	28	8	15	-6	-8	-3	-8	Clear
Jan	29	8	15	-7	-9	-4	-9	Clear
Jan	30	8	15	-8	-10	-5	-10	Clear
Jan	31	8	15	-9	-11	-6	-11	Clear

(Appendix I-A - cont'd)

3. FALLIS (Gray-wooded-soil zone)

Variety	Replicate				Mean and S.D.		Variation range	
	1	2	3	4			Min.	Max.
C-16	35	47	32	32	37	$\pm$ 2	35	39
Canus	25	35	40	29	32	2	30	34
Carter's E.F.	59	48	73	47	57	12	45	69
E. Dewey	79	58	66	90	73	14	59	87
E. Ohio	94	67	64	47	68	19	49	87
I. Cobbler	14	15	39	24	23	12	11	35
Katahdin	48	35	43	38	41	2	39	43
Latowski	26	29	37	32	31	1	30	32
Manota	60	27	34	43	41	14	27	55
Netted Gem	54	33	44	61	48	12	36	60
50-3	34	23	37	56	38	14	24	52
50-7	16	19	16	22	18	3	15	21
177	16	16	20	16	17	2	15	19
Penner's Blue	50	57	33	41	45	14	31	59
Warba	74	38	62	47	55	21	34	76
Mean					42	$\pm$ 19	23	71

(Appendix I - cont'd)

B. Analysis of variance. *)

Source of variation	Sums of squares	Degrees of freedom	Mean square	F	Significance 1% 5%
Total variation	886.48	179			
Varieties	515.51	14	36.82	7.12**	2.80
Locations	25.02	2	12.51	2.42	2.95
Var. x Loc.	144.69	28	5.17	3.70**	1.85
Replicates	16.58	3	5.53	3.91*	3.93 2.68
Error	184.68	132	1.40		

L.S.D. at the one percent level for "varieties" = 22 p.p.m.
fresh weight.

*) The data have been processed as mgm. solanine per 100 gm.
fresh tuber, which represents 1/10 p.p.m. statistic.

APPENDIX II

(DATA ON EXPERIMENT II)

A. Solar radiation and storage effects upon solanine concentrations of eight potato varieties. Data as p.p.m. fresh tuber weight.

1. Solar radiation effect

Variety	Replicate	Exposure duration								
		Nil			8 hours			72 hours		
		B*	E	F	B	E	F	B	E	F
C-16	1	21	19	35	82	58	70	120	118	134
	2	26	24	32	61	78	37	159	87	152
E. Dewey	1	50	57	79	129	97	114	350	227	249
	2	52	96	90	98	114	147	305	186	284
E. Ohio	1	79	51	94	106	97	115	313	210	213
	2	82	67	47	148	143	80	235	228	192
I. Cobbler	1	13	16	14	33	35	44	51	78	38
	2	24	32	24	46	29	28	62	81	23
Katahdin	1	24	32	48	66	48	76	134	130	156
	2	32	47	38	50	59	60	161	104	161
Netted Gem	1	55	43	53	129	108	97	396	268	213
	2	65	72	61	172	89	68	257	238	316
50-3	1	26	16	34	36	29	59	97	134	139
	2	17	27	56	57	37	39	130	75	170
177	1	15	17	16	36	30	47	169	86	197
	2	27	28	16	58	51	82	298	165	121

* B, E, F designates the locations at which potatoes were grown: Brooks, Edmonton, Fallis, respectively.

Table 1

(a) Summary of results

—The following table shows the results of the experiments conducted during the year 1911, and is intended to give a general idea of the work done during the year.

—The following table shows the results of the experiments conducted during the year 1911, and is intended to give a general idea of the work done during the year.

(b) Summary of results

Experiment No.			Date			Time			Result		
No.	1	2	No.	1	2	No.	1	2	No.	1	2
1	10	10	1	10	10	1	10	10	1	10	10
2	10	10	2	10	10	2	10	10	2	10	10
3	10	10	3	10	10	3	10	10	3	10	10
4	10	10	4	10	10	4	10	10	4	10	10
5	10	10	5	10	10	5	10	10	5	10	10
6	10	10	6	10	10	6	10	10	6	10	10
7	10	10	7	10	10	7	10	10	7	10	10
8	10	10	8	10	10	8	10	10	8	10	10
9	10	10	9	10	10	9	10	10	9	10	10
10	10	10	10	10	10	10	10	10	10	10	10
11	10	10	11	10	10	11	10	10	11	10	10
12	10	10	12	10	10	12	10	10	12	10	10
13	10	10	13	10	10	13	10	10	13	10	10
14	10	10	14	10	10	14	10	10	14	10	10
15	10	10	15	10	10	15	10	10	15	10	10
16	10	10	16	10	10	16	10	10	16	10	10
17	10	10	17	10	10	17	10	10	17	10	10
18	10	10	18	10	10	18	10	10	18	10	10
19	10	10	19	10	10	19	10	10	19	10	10
20	10	10	20	10	10	20	10	10	20	10	10

—The following table shows the results of the experiments conducted during the year 1911, and is intended to give a general idea of the work done during the year.

(Appendix II-A - cont'd)

2. Storage effects upon tubers exposed to solar radiation.

Variety	Replicate	Exposure duration								
		Nil			8 hours			72 hours		
		B*	E	F	B	E	F	B	E	F
C-16	1	22	18	26	102	40	61	112	118	186
	2	31	22	22	83	46	41	168	131	123
E. Dewey	1	42	81	100	143	85	107	375	355	337
	2	66	98	73	176	120	160	238	375	279
E. Ohio	1	77	60	68	185	148	127	275	251	214
	2	47	87	113	102	119	95	228	196	361
I. Cobbler	1	24	29	17	89	31	29	52	63	53
	2	23	31	26	59	27	38	65	38	86
Katahdin	1	46	20	44	85	49	70	218	265	180
	2	51	32	61	117	57	50	121	238	122
Netted Gem	1	32	46	27	140	87	105	265	350	426
	2	32	67	46	170	122	76	251	469	369
50-3	1	15	17	15	47	59	49	93	90	88
	2	14	19	13	68	38	41	62	42	128
177	1	13	17	12	62	46	37	95	123	155
	2	14	19	11	86	41	59	145	91	183

* See footnote under A-1.

(Appendix II - cont'd)

B. Analysis of variance* on solanine data in section A.

Source of variation	S.S.	D.F.	M.S.	F	Significance	
					1%	5%
Total variation	39.821	287				
Replicates	0.038	1	0.038	2.53		18.51
Locations	0.105	2	0.053	3.50		19.00
Error (1)	0.030	2	0.015			
Storage	0.002	1	0.002	1		
Varieties	12.692	7	1.813	151.08**	2.75	
Treatments	21.330	2	10.665	180.76**	18.00	
Loc. x Stor.	0.009	2	0.005	1		
Loc. x Var.	0.277	14	0.020	1		
Loc. x Treat.	0.359	4	0.090	1.53		6.39
Stor. x Var.	0.299	7	0.043	1		
Stor. x Treat.	0.099	2	0.050	1		
Var. x Treat.	1.318	14	0.094	1.60		5.87
Loc.xStor.xVar.	0.289	14	0.021	1.75		1.75
Loc.xStor.xTreat.	0.237	4	0.059	4.92**	3.43	
Loc.xVar.xTreat.	0.450	28	0.016	1.34		1.53
Stor.xVar.xTreat.	0.256	14	0.018	1.52		1.75
Error (2)	2.031	169	0.012			

* Solanine data subjected to logarithmic transformation.

Significance: L.S.D. = 0.097 (treatment means)

<u>Exposure</u>	<u>Mean</u>	<u>Difference</u>
Nil	1.525	
8 hr.	1.840	0.315
72 hr.	2.191	0.351

(Appendix II - cont'd)

C. Percentage loss of weight of stored tuber samples
in section A-2.

Variety	Replicate	Exposure duration								
		Nil			8 hours			72 hours		
		B*	E	F	B	E	F	B	E	F
C-16	1	2.0	2.5	2.2	1.7	2.1	2.4	2.4	3.3	2.8
	2	1.7	2.9	2.0	1.7	1.8	1.6	3.3	3.4	2.7
E. Dewey	1	1.2	2.0	1.9	1.4	1.7	1.8	2.8	1.9	2.2
	2	1.8	2.4	1.9	1.4	1.6	1.1	2.3	1.7	1.8
E. Ohio	1	1.0	2.2	2.0	1.4	2.5	1.6	2.1	2.2	2.1
	2	1.8	1.7	1.7	1.6	1.9	3.1	2.9	2.8	2.5
I. Cobbler	1	1.0	1.8	1.3	0.8	1.6	0.9	1.9	2.8	1.6
	2	1.5	1.5	0.9	1.1	1.4	1.2	1.6	2.0	2.0
Katahdin	1	2.3	2.1	1.8	1.5	1.7	1.8	3.5	3.3	2.4
	2	1.8	2.1	2.0	1.8	1.6	1.8	3.2	2.5	2.9
Netted Gem	1	1.6	2.2	2.2	3.8	1.3	1.5	2.0	1.6	2.2
	2	1.3	1.5	2.0	1.3	1.4	1.4	2.2	2.1	1.8
50-3	1	2.5	3.4	7.4	1.8	2.0	1.9	4.3	2.2	4.8
	2	2.1	3.1	2.9	2.6	2.1	3.2	2.7	4.1	4.2
177	1	2.5	3.3	3.6	2.3	3.2	1.5	4.3	5.3	3.5
	2	2.6	4.0	3.5	2.1	2.2	2.8	5.0	3.3	3.8

* See footnote under A-1.

(Appendix II - cont'd)

- D. Temperature and relative humidity data recorded during the solar-radiation exposures and in the storage.

1. Solar-radiation data

Exposure	Temperature range (°F.)	Duration hours	Humidity range (%)	Duration hours
8 hours	48-50	$\frac{1}{2}$	40-60	$3\frac{1}{2}$
	50-55	$1\frac{1}{2}$	60-80	2
	55-60	$2\frac{1}{2}$	Above 80	$2\frac{1}{2}$
	60-63	$3\frac{1}{2}$		
72 hours	33-35	11	35-40	$4\frac{1}{2}$
	35-40	9	40-60	19
	40-45	8	60-80	21
	45-50	17	Above 80	$27\frac{1}{2}$
	50-55	9		
	55-60	6		
	60-63	10		

2. Storage temperature and humidity fluctuation

Temperature	Humidity
44-48° F.	80-85%

APPENDIX III

(DATA ON EXPERIMENT III)

Tuber sample weights (gms.), percentage weight loss of stored tubers, and corresponding solanine concentrations as p.p.m. fresh weight.

Variety	Photoperiod	Repl.	Storage duration					
			N i l			8 w e e k s		
			Sample wt. gms.	Sola- nine p.p.m.	Sample wt. gms.	Loss of wt. gms.	%	Sola- nine p.p.m.
Netted Gem	Long (control)	1	825	9	595	13	2.2	19
		2	790	12	815	23	2.8	20
		3	715	16	864	25	2.9	17
	Short	1	525	31	665	15	2.3	13
		2	585	26	445	8	1.8	20
		3	491	21	563	12	2.1	25
Katahdin	Long (control)	1	1020	24	1250	19	2.3	21
		2	835	22	1367	18	1.4	13
		3	965	16	902	17	1.3	12
	Short	1	483	37	692	13	3.9	35
		2	552	22	528	10	3.2	29
		3	728	26	875	14	2.8	25

Table 1

(continued from page 14)

Notes: 1. All data are preliminary and subject to change. 2. Data are based on the results of the analysis of variance. 3. Data are based on the results of the analysis of variance. 4. Data are based on the results of the analysis of variance.

Table 1 (continued)

No.	Factor A		Factor B		Interaction	Total
	Level	Mean	Level	Mean		
1	1.0	1.0	1.0	1.0	1.0	1.0
2	1.0	1.0	1.0	1.0	1.0	1.0
3	1.0	1.0	1.0	1.0	1.0	1.0
4	1.0	1.0	1.0	1.0	1.0	1.0
5	1.0	1.0	1.0	1.0	1.0	1.0
6	1.0	1.0	1.0	1.0	1.0	1.0
7	1.0	1.0	1.0	1.0	1.0	1.0
8	1.0	1.0	1.0	1.0	1.0	1.0
9	1.0	1.0	1.0	1.0	1.0	1.0
10	1.0	1.0	1.0	1.0	1.0	1.0
11	1.0	1.0	1.0	1.0	1.0	1.0
12	1.0	1.0	1.0	1.0	1.0	1.0
13	1.0	1.0	1.0	1.0	1.0	1.0
14	1.0	1.0	1.0	1.0	1.0	1.0
15	1.0	1.0	1.0	1.0	1.0	1.0
16	1.0	1.0	1.0	1.0	1.0	1.0
17	1.0	1.0	1.0	1.0	1.0	1.0
18	1.0	1.0	1.0	1.0	1.0	1.0
19	1.0	1.0	1.0	1.0	1.0	1.0
20	1.0	1.0	1.0	1.0	1.0	1.0
21	1.0	1.0	1.0	1.0	1.0	1.0
22	1.0	1.0	1.0	1.0	1.0	1.0
23	1.0	1.0	1.0	1.0	1.0	1.0
24	1.0	1.0	1.0	1.0	1.0	1.0
25	1.0	1.0	1.0	1.0	1.0	1.0
26	1.0	1.0	1.0	1.0	1.0	1.0
27	1.0	1.0	1.0	1.0	1.0	1.0
28	1.0	1.0	1.0	1.0	1.0	1.0
29	1.0	1.0	1.0	1.0	1.0	1.0
30	1.0	1.0	1.0	1.0	1.0	1.0
31	1.0	1.0	1.0	1.0	1.0	1.0
32	1.0	1.0	1.0	1.0	1.0	1.0
33	1.0	1.0	1.0	1.0	1.0	1.0
34	1.0	1.0	1.0	1.0	1.0	1.0
35	1.0	1.0	1.0	1.0	1.0	1.0
36	1.0	1.0	1.0	1.0	1.0	1.0
37	1.0	1.0	1.0	1.0	1.0	1.0
38	1.0	1.0	1.0	1.0	1.0	1.0
39	1.0	1.0	1.0	1.0	1.0	1.0
40	1.0	1.0	1.0	1.0	1.0	1.0
41	1.0	1.0	1.0	1.0	1.0	1.0
42	1.0	1.0	1.0	1.0	1.0	1.0
43	1.0	1.0	1.0	1.0	1.0	1.0
44	1.0	1.0	1.0	1.0	1.0	1.0
45	1.0	1.0	1.0	1.0	1.0	1.0
46	1.0	1.0	1.0	1.0	1.0	1.0
47	1.0	1.0	1.0	1.0	1.0	1.0
48	1.0	1.0	1.0	1.0	1.0	1.0
49	1.0	1.0	1.0	1.0	1.0	1.0
50	1.0	1.0	1.0	1.0	1.0	1.0

APPENDIX IV

(DATA ON EXPERIMENT IV)

A. Complete data on solanine analyses of tubers in
illuminated and dark storage.
Data as p.p.m. fresh weight.

		Dark storage				Illuminated storage			
Storage duration *	Repl.	Katahdin		N. Gem		Katahdin		N. Gem	
		Temperature				Temperature			
		Low	High	Low	High	Low	High	Low	High
0 (control)	1	23	23	40	40	23	23	40	40
	2	20	20	16	16	20	20	16	16
	3	33	33	27	27	33	33	27	27
2	1	30	52	38	41	20	97	66	55
	2	33	39	21	42	46	74	97	64
	3	66	43	39	66	40	115	106	88
4	1	38	61	44	53	26	86	135	92
	2	35	52	49	72	42	120	103	82
	3	47	69	27	34	57	92	129	103
6	1	45	55	64	60	41	86	163	144
	2	28	40	47	89	68	125	111	92
	3	35	54	92	52	52	89	159	113
8	1	30	70	80	87	71	129	191	98
	2	40	51	51	104	81	108	177	107
	3	47	65	98	65	59	141	143	148

* Intervals for dark storage in weeks; for illuminated storage in days.

(Appendix IV - cont'd)

B. Analysis of variance.

1. Tuber samples stored in absence of light.

Source of variation	S.S.	D.F.	M.S.	F	Significance	
					1%	5%
Total variation	251.94	59				
Varieties	15.40	1	15.40	1.98		4.10
Replicates	6.27	2	3.14	1.67		3.25
Sampling dates	102.51	4	25.63	3.30*	3.86	2.62
Temperature	14.21	1	14.21	7.56**	7.35	
Var. x Dates	31.03	4	7.76	4.13**	3.86	
Var. x Temp.	0.64	1	0.64	<1		
Dates x Temp.	5.86	4	1.21	<1		
Var. x Dates x Temp.	4.57	4	1.14	<1		
Error	71.45	38	1.88			

2. Tuber samples in illuminated storage.

Source of variation	S.S.	D.F.	M.S.	F	Significance	
					1%	5%
Total variation	1,208.85	59				
Varieties	139.54	1	139.54	9.95**	7.35	
Replicates	8.98	2	4.49	1.39		3.25
Sampling dates	625.65	4	156.41	11.16**	3.86	
Temperature	11.70	1	11.70	<1		
Var. x Dates	56.09	4	14.02	3.54*	3.86	2.62
Var. x Temp.	184.80	1	184.80	46.67**	7.35	
Dates x Temp.	8.23	4	2.06	<1		
Var. x Dates x Temp.	51.11	4	12.78	3.96**	3.86	
Error	122.75	38	3.23			

(continued - 1995/1996)

Table 1. Summary of the 1995/1996 season

Table 1. Summary of the 1995/1996 season

Station	Area	Depth	Time	Temperature	Salinity
				(°C)	(PSU)
01.01	10.0	10.0	1	10.0	10.0
02.01	10.0	10.0	2	10.0	10.0
03.01	10.0	10.0	3	10.0	10.0
04.01	10.0	10.0	4	10.0	10.0
05.01	10.0	10.0	5	10.0	10.0
06.01	10.0	10.0	6	10.0	10.0
07.01	10.0	10.0	7	10.0	10.0
08.01	10.0	10.0	8	10.0	10.0
09.01	10.0	10.0	9	10.0	10.0
10.01	10.0	10.0	10	10.0	10.0
11.01	10.0	10.0	11	10.0	10.0
12.01	10.0	10.0	12	10.0	10.0
13.01	10.0	10.0	13	10.0	10.0
14.01	10.0	10.0	14	10.0	10.0
15.01	10.0	10.0	15	10.0	10.0
16.01	10.0	10.0	16	10.0	10.0
17.01	10.0	10.0	17	10.0	10.0
18.01	10.0	10.0	18	10.0	10.0
19.01	10.0	10.0	19	10.0	10.0
20.01	10.0	10.0	20	10.0	10.0
21.01	10.0	10.0	21	10.0	10.0
22.01	10.0	10.0	22	10.0	10.0
23.01	10.0	10.0	23	10.0	10.0
24.01	10.0	10.0	24	10.0	10.0
25.01	10.0	10.0	25	10.0	10.0
26.01	10.0	10.0	26	10.0	10.0
27.01	10.0	10.0	27	10.0	10.0
28.01	10.0	10.0	28	10.0	10.0
29.01	10.0	10.0	29	10.0	10.0
30.01	10.0	10.0	30	10.0	10.0
31.01	10.0	10.0	31	10.0	10.0
01.02	10.0	10.0	1	10.0	10.0
02.02	10.0	10.0	2	10.0	10.0
03.02	10.0	10.0	3	10.0	10.0
04.02	10.0	10.0	4	10.0	10.0
05.02	10.0	10.0	5	10.0	10.0
06.02	10.0	10.0	6	10.0	10.0
07.02	10.0	10.0	7	10.0	10.0
08.02	10.0	10.0	8	10.0	10.0
09.02	10.0	10.0	9	10.0	10.0
10.02	10.0	10.0	10	10.0	10.0
11.02	10.0	10.0	11	10.0	10.0
12.02	10.0	10.0	12	10.0	10.0
13.02	10.0	10.0	13	10.0	10.0
14.02	10.0	10.0	14	10.0	10.0
15.02	10.0	10.0	15	10.0	10.0
16.02	10.0	10.0	16	10.0	10.0
17.02	10.0	10.0	17	10.0	10.0
18.02	10.0	10.0	18	10.0	10.0
19.02	10.0	10.0	19	10.0	10.0
20.02	10.0	10.0	20	10.0	10.0
21.02	10.0	10.0	21	10.0	10.0
22.02	10.0	10.0	22	10.0	10.0
23.02	10.0	10.0	23	10.0	10.0
24.02	10.0	10.0	24	10.0	10.0
25.02	10.0	10.0	25	10.0	10.0
26.02	10.0	10.0	26	10.0	10.0
27.02	10.0	10.0	27	10.0	10.0
28.02	10.0	10.0	28	10.0	10.0
29.02	10.0	10.0	29	10.0	10.0
30.02	10.0	10.0	30	10.0	10.0
31.02	10.0	10.0	31	10.0	10.0

Table 1. Summary of the 1995/1996 season

Station	Area	Depth	Time	Temperature	Salinity
				(°C)	(PSU)
01.02	10.0	10.0	1	10.0	10.0
02.02	10.0	10.0	2	10.0	10.0
03.02	10.0	10.0	3	10.0	10.0
04.02	10.0	10.0	4	10.0	10.0
05.02	10.0	10.0	5	10.0	10.0
06.02	10.0	10.0	6	10.0	10.0
07.02	10.0	10.0	7	10.0	10.0
08.02	10.0	10.0	8	10.0	10.0
09.02	10.0	10.0	9	10.0	10.0
10.02	10.0	10.0	10	10.0	10.0
11.02	10.0	10.0	11	10.0	10.0
12.02	10.0	10.0	12	10.0	10.0
13.02	10.0	10.0	13	10.0	10.0
14.02	10.0	10.0	14	10.0	10.0
15.02	10.0	10.0	15	10.0	10.0
16.02	10.0	10.0	16	10.0	10.0
17.02	10.0	10.0	17	10.0	10.0
18.02	10.0	10.0	18	10.0	10.0
19.02	10.0	10.0	19	10.0	10.0
20.02	10.0	10.0	20	10.0	10.0
21.02	10.0	10.0	21	10.0	10.0
22.02	10.0	10.0	22	10.0	10.0
23.02	10.0	10.0	23	10.0	10.0
24.02	10.0	10.0	24	10.0	10.0
25.02	10.0	10.0	25	10.0	10.0
26.02	10.0	10.0	26	10.0	10.0
27.02	10.0	10.0	27	10.0	10.0
28.02	10.0	10.0	28	10.0	10.0
29.02	10.0	10.0	29	10.0	10.0
30.02	10.0	10.0	30	10.0	10.0
31.02	10.0	10.0	31	10.0	10.0
01.03	10.0	10.0	1	10.0	10.0
02.03	10.0	10.0	2	10.0	10.0
03.03	10.0	10.0	3	10.0	10.0
04.03	10.0	10.0	4	10.0	10.0
05.03	10.0	10.0	5	10.0	10.0
06.03	10.0	10.0	6	10.0	10.0
07.03	10.0	10.0	7	10.0	10.0
08.03	10.0	10.0	8	10.0	10.0
09.03	10.0	10.0	9	10.0	10.0
10.03	10.0	10.0	10	10.0	10.0
11.03	10.0	10.0	11	10.0	10.0
12.03	10.0	10.0	12	10.0	10.0
13.03	10.0	10.0	13	10.0	10.0
14.03	10.0	10.0	14	10.0	10.0
15.03	10.0	10.0	15	10.0	10.0
16.03	10.0	10.0	16	10.0	10.0
17.03	10.0	10.0	17	10.0	10.0
18.03	10.0	10.0	18	10.0	10.0
19.03	10.0	10.0	19	10.0	10.0
20.03	10.0	10.0	20	10.0	10.0
21.03	10.0	10.0	21	10.0	10.0
22.03	10.0	10.0	22	10.0	10.0
23.03	10.0	10.0	23	10.0	10.0
24.03	10.0	10.0	24	10.0	10.0
25.03	10.0	10.0	25	10.0	10.0
26.03	10.0	10.0	26	10.0	10.0
27.03	10.0	10.0	27	10.0	10.0
28.03	10.0	10.0	28	10.0	10.0
29.03	10.0	10.0	29	10.0	10.0
30.03	10.0	10.0	30	10.0	10.0
31.03	10.0	10.0	31	10.0	10.0

(Appendix IV - cont'd)

C. Mean percentage of tuber weight loss during the storage.

Storage duration *	Dark storage				Illuminated storage			
	Katahdin		N. Gem		Katahdin		N. Gem	
	Temperature				Temperature			
	Low	High	Low	High	Low	High	Low	High
2	1.2	1.9	1.9	3.2	0.9	1.6	1.1	1.7
4	1.8	2.4	2.4	4.4	1.2	2.8	1.7	2.4
6	2.0	3.2	2.8	5.6	1.6	2.3	2.3	3.1
8	2.7	4.4	3.5	6.3	1.8	2.9	2.3	2.9

* Intervals for dark storage in weeks; for illuminated storage in days.

D. Temperature and humidity fluctuations.

	Storage chamber			
	Low temperature		High temperature	
	Dark	Illuminated	Dark	Illuminated
Temperature (°F)	44-48	51-53	56-62	64-68
Humidity (% R.H.)	80-85	70-75	30-40	20-25

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APPENDIX V

(DATA ON EXPERIMENT V)

A. Solanine and solanidine determinations in potato peels and tuber flesh of bitter Netted Gem potatoes.

Data as p.p.m. fresh weight.

Tuber region	Repl.	Sample weight			Concentration		
		Whole tuber gms.	Peels		p.p.m.		
			gms.	%	Sola- nine	Solani- dine	Total solanine
(1) Potato peels	1	826.4	88.5	10.71	878	421	1299
	2	815.8	118.5	14.52	995	285	1280
	3	815.0	121.9	14.96	1079	292	1371
	4	833.4	129.5	15.54	1072	355	1427
Mean				13.93	1006	338	1344
(2) Tuber flesh	1	826.4	737.9	89.29	394	63	457
	2	815.8	697.3	85.48	445	38	483
	3	815.0	693.1	85.04	337	62	399
	4	833.4	703.9	84.46	387	54	441
Mean				86.07	391	54	445

(Appendix V - cont'd)

B. Minimum and maximum temperature readings
during the solar radiation exposure.

Days of exposure	Temperature Degrees of Fahrenheit	
	Min.	Max.
1	26	42
2	36	49
3	34	55
4	32	59
5	41	59
6	28	56
7	42	51

APPENDIX VIMaintenance of Prepared Tuber Samples

Experiment IV originally was designed to contain four replicates. However, because of the large number of samples involved in tuber material from a single growing season, and also because of the transfer of laboratory equipment to new premises, analyses of one replicate had to be postponed. When the samples of this replicate were defrosted for analysis after a 12-month storage period, the minced material showed an extreme blackening. Compared to the analyses of the first three replicates carried out after seven to eight months of frozen storage, approximately 30 to 50 percent of the solanine appeared to be lost, as shown in Table VI-A. The inclusion of such results

Table VI-A. Solanine concentrations in tissue of samples from 6 to 8 months frozen storage compared to samples stored for 12 months.

Storage duration (illuminated tubers)	Replicate	Netted Gem		Katahdin	
		Temperature		Temperature	
		Low	High	Low	High
4 weeks	1-3 (mean)	122	92	42	99
	4	85	62	20	42
8 weeks	1-3 (mean)	170	118	70	126
	4	114	73	37	79

would introduce a serious error, and therefore the data from the fourth replicate were omitted. Fortunately, the remaining three replicates sufficed to indicate the gross effects of treatments imposed in the particular trial.

Earlier in the work, in certain samples from other trials, attempts were made to prevent undesirable enzymatic activities in the frozen samples, and particularly during the maceration process. Although only a slight decrease in solanine concentration was noted in samples left for 20 hours at room temperature, the addition of glacial acetic acid to 0.5 percent, or thiourea to 0.01 M concentration, appeared to prevent blackening of the minced tissue, due to inhibition of oxidative enzymes. Some of the samples treated in this manner were kept in quick-frozen state without traces of blackening for as long as 12 months.

Table VI-B. The effect of antioxidation treatments upon the solanine content of potato tuber tissue.
(Three lots of Netted Gem potatoes of different origin analyzed after 2 months of frozen storage.)

Treatment	Solanine, p.p.m. fresh weight		
Minced, frozen immediately	44	94	64
Minced, frozen after 20 hrs. at room temperature	32	83	58
Thiourea added to 0.01 M conc.	50	102	68
Glac. acetic acid added to 0.5%	61	105	72

Samples which were not frozen until 20 hours after the mincing generally showed slightly lower levels of solanine, as well as extreme blackening of ground tissues, in all probability due to enzymatic oxidation and to melanin formation. As the addition of acetic acid during the maceration prevented such a process and appeared to result in slightly improved extraction, this procedure was adopted for sample preparation; but unfortunately too late to be useful in the present investigation, with the exception of tuber samples used in Experiment V.

In addition to the effects noted above, experience showed that the macerated tissue should be stored in relatively tightly closed tinned or glass containers, since moisture losses in storage tend to increase the readings.

APPENDIX VIINotes on Field Sampling Technique

Single-hill sampling, although statistically unsuitable, was adopted for the following reasons:

(1) To eliminate during the actual harvest any prolonged light exposure, which would be more likely to occur when harvesting all hills from a single replicate, since it would be necessary to spread out the tuber material and select four statistically appropriate samples (one for Experiment I and three for Experiment II). This compares with the very brief exposure-interval necessary when a single entire hill is harvested as a sample.

(2) To ensure selection of the necessary samples from completely healthy hills. Leaf roll virus infection was widespread and serious in many varieties.

(3) To secure tubers from a uniform depth, and to exclude any tubers produced near the soil surface. This did not appear to be feasible by the use of other sampling procedures.

The effect of geographical locations was considered to be adequately tested by four randomized replications, and the biometrical inadequacies of the single-hill sampling technique were thought to be less serious than those involved in the excessive handling and exposure, had a large number of hills to be harvested for bulking to provide a sample more representative of the plot.

Observation of the data in Experiment I (see Appendix I-A) indicates that the significant F value for replicates (Appendix I-B) expresses the variance between, rather than within, a single location. This overall effect is attributed to the influence of transport conditions and a delay in sample preparations (see text, p. 33). Moreover, the single-hill technique was successfully employed in earlier work, when a negligible variation was observed in over 150 tuber samples of three potato varieties prepared for analysis within a few hours after harvest. In the majority of samples, the variation was frequently less than the error of the analytical procedure (62).

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