# THE USE OF GIBBERELLIC ACID (GA) AND N-DIMETHYLAMINOSUCCINAMIC ACID (B9) IN THE TESTING OF SEED POTATOES FOR VIRUS INFECTION

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*Zusammenfassung, Résumé p. 150*

SUMMARY

A method is described for the breaking of dormancy of potato tuber buds and for regulating the growth of the subsequent plants, particularly for use in inspection for the incidence of virus diseases. The method consists of dipping excised eyes in gibberellic acid (GA) solution, usually 1 p.p.m., for 10 minutes. In varieties with a naturally long dormant period the concentration can be increased to 5 p.p.m.; when dormancy is only superficial and slender growth is anticipated, 100 to 5,000 p.p.m. N-dimethylaminosuccinamic acid (B9 or B995) can be added to suppress excessive stem elongation. The implications of the method and its advantages over the use of rindite are discussed.

## 1. INTRODUCTION

In The Netherlands, field inspection of seed potato crops for virus disease is followed by laboratory tests on tuber samples.

1. Heel-ends of tubers are submitted to the Igel-Lange test for leaf-roll (ARENZ and HUNNIUS, 1963).
2. The incidence of viruses A and Y is determined by inoculating isolated leaves of "A6" hybrids (*Solanum tuberosum* "Aquila" × *S. demissum*) with sap from sprouts (KÖHLER, 1953).
3. Plants grown from excised eyes of sample tubers are visually inspected for virus symptoms. Leaves of these plants may then be subjected to the "A6" and serological tests (DE BOKX, 1964; KELLER and BÉRCES, 1966).

Methods 2 and 3 require tubers the dormancy of which has been broken. Until recently this was done by treating them with rindite (ethylene chlorohydrin : dichloroethylene : carbon tetrachloride = 7:2:1).

The main disadvantage of this method is that, in our experience, the heel ends of rindite-treated tubers cannot be used safely in the Igel-Lange test because of callose

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formation unconnected with the presence of leaf-roll virus. This difficulty, although it does not always arise (MÜNSTER, 1965), forces to take duplicate samples. In addition, there is a rather narrow margin between the dormancy-breaking and phytotoxic dosages of rindite and, particularly with freshly harvested tubers, rotting can occur readily. Finally, treatment with a toxic and evil-smelling gas is unpleasant and the method both time- and labour-consuming.

Because of these drawbacks, another method has been developed for breaking the dormancy of potato tubers, which permits of the application of all the tests to the same sample and is both safe and rapid. It is based on the dormancy-breaking action of the non-toxic growth regulator gibberellic acid (GA) (BRUINSMA, 1962). Because GA may, as well as breaking dormancy, induce excessive stem elongation, it may be applied in combination with the growth retardant, N-dimethylaminosuccinamic acid (B9 or B995) (KRUG and WIGGER, 1964), which suppresses growth without interfering with the breaking of dormancy. Some of the results obtained with this method were reported earlier by BAKKER, BRUINSMA and SINNEMA (1966).

## 2. MATERIALS AND METHODS

Following preliminary experiments in 1964, 1,369 samples of tubers from seed crops of 45 varieties, grown in North Holland and the North East Polder, were used in the late summer and autumn of 1965 for experimental and routine assays. Most samples were of the varieties *Alpha* (200), *Bintje* (274), *Doré* (185), *Eersteling* (92), *Eigenheimer* (47), *Furore* (77), *Irene* (70), *Libertas* (24) and *Sirtema* (79). This paper is mainly based upon observations of this material, modified in the light of results obtained in 1966, in which year the GA method completely replaced the rindite-method in the work of the regional Inspection Services of North Holland and of the North East Polder and Flevoland.

The following data were collected for each tuber sample: variety, quality class, origin; dates of chemical haulm destruction, sampling treatment, planting, emergence and inspection for virus diseases; regularity and rate of emergence and leaf development; growth rate; appearance of virus symptoms; results of serological and "A6" tests.

Most of the samples were divided into two, one part being submitted to the normal rindite treatment, the other to one or more GA treatments.

With the rindite method whole tubers were treated with 0.8–1.0 ml rindite per kg tubers for 2 or 3 days in closed containers at 26–28 °C. They were then pre-sprouted under humid conditions at 20–25 °C for about 14 days and the sprouted eyes from the rose-end cut out with a hemispherical butterknife. These were placed in soil in a greenhouse and provided plants suitable for testing in about 4 weeks.

With the GA treatment the eyes were excised without pre-sprouting, immersed for 10 minutes in a solution of GA, normally 1 p.p.m., and planted; these produced sizable plants in 4–5 weeks. In some of the experiments, excised eyes were treated with solutions of B9.

The gibberellic acid used was "Berelex" powder (I.C.I.), containing 90% $A_3$, 10% $A_4$ + $A_7$, and a trace of $A_1$. The N-dimethylaminosuccinamic acid (Naugatuck Chemical) was obtained from Ligtermoet Chemie N.V., and the wettable powder formulations are to be preferred to the 5% solution.

Gibberellic acid was estimated with an accuracy of about 10% from the size of spots on paper chromatograms developed with butyl-acetate and detected by spraying with 3% $H_2SO_4$ in methanol (PODOJIL and ŠEVCÍK, 1960).

N-dimethylaminosuccinamic acid was determined by a modification of a method supplied by Naugatuck Chemical. The decomposition product of B9 in alkaline solution with zinc, dimethyl hydrazine, was distilled into a phosphate citrate buffer of pH 6.3. The intensity of the red colour, which developed 30 min after addition of trisodium pentacyanoaminoferrate (Aldrich Chemical), was measured in a Unicam SP 600 spectrophotometer at 488 nm and compared with a standard curve between 10 and 130 p.p.m. B9.

## 3. RESULTS

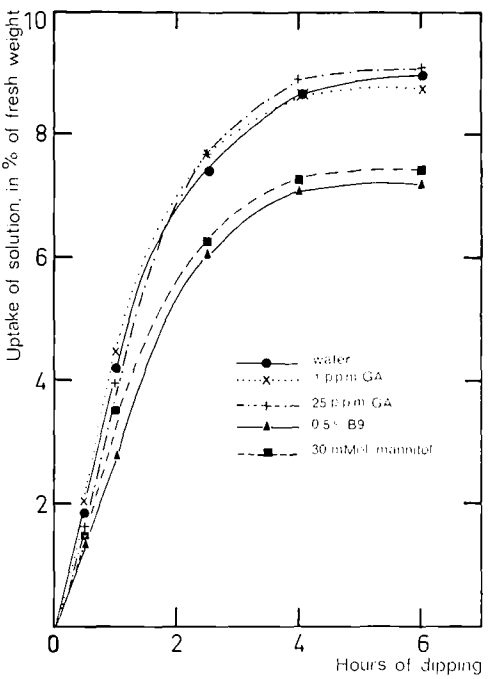
### 3.1. The dipping treatment

Dipping of whole, undamaged tubers showed that the resistance of the periderm to penetration of the regulators GA and B9 offered serious difficulties. Even very young tubers with thin, tender skins often hardly responded to such treatments, except at higher concentrations of GA which also caused unacceptable growth disturbances. In contrast to a number of results mentioned in the literature (CHOUDHURI and GHOSE, 1963; GRECHUSHNIKOV, KIRYUKHIN, SEREBRENIKO and TEKTONIDI, 1964; LADYGINA, 1964; SEMERDŽJAN and AVAKJAB, 1965; SMITH and RAPPAPORT, 1965), we found that treatment of whole, undamaged tubers did not give reproduceable results.

Superficial wounding of the tubers may greatly improve the penetration of solutions (SLOMNYCKI and RYLSKI, 1964) but wounding on a nail-bed prior to dipping showed that the margin between effective and malforming dosages was too narrow to permit of practical application on a large range of varieties with different degrees of dormancy and susceptibility. Therefore treatment of excised eyes is essential to a large scale routine method.

The rate of absorption of solution by excised eyes is shown in Fig. 1. The minute amounts of GA used, from 1 to 25 p.p.m., affect neither the permeability nor the osmotic value, the rates of absorption being indistinguishable from those of distilled water. Solutions of B9, if prepared from the 50% wettable powder, either at the original pH (3.7) or when neutralized, give about the same absorption curves as equimolar solutions of mannitol, showing that B9 interferes no more with the uptake of solution than does GA, except for its higher osmotic value. The absorption of B9 solutions prepared from the 5% liquid formulation, however, shows abnormalities due to the surface-active agent present in this product.

Fig. 1. Absorption of solutions by excised eyes. See text.




Uptake of solution, in % of fresh weight – *Aufnahme der Lösung in % des Frischgewichtes – prélèvement de solution en % de poids frais*
Hours of dipping – *Eintauchzeit (Stunden) – heures de trempage*
Water – *Wasser – eau*

*Abb. 1. Aufnahme der Lösungen durch ausgestochene Augenstücke. Siehe Text.*
*Fig. 1. Absorption de solution par les yeux excisés. Voir texte.*

During the first few hours uptake proceeds at a constant rate, then gradually levels off at about 10% of the orginal fresh weight of the excised eyes. With the usual dipping time of 10 minutes, the excised eyes absorb only up to 1% of their weight, and upon transfer from one solution to another (e.g. from 1 p.p.m. GA to 0.5% B9), the absorption process continues without any demonstrable release of the previously absorbed regulator. It is immaterial in this respect, whether the two regulators are combined in one solution or are applied separately.

Investigations of the absorption rates of the regulators themselves, relative to the absorption rate of the water, were carried out with samples of 20 discs of tuber tissue, 10 mm in diameter and 5 mm thick, shaken for 15 minutes in 100 ml solution. The samples were replaced several times by fresh ones before the final regulator concentrations in the solutions were determined as described under section 2. (Methods). The results, some of which are summarized in Table 1 and 2, show that the concentration

Table 1. Effect of repeated dipping on GA concentration in the solution

| | *GA concentration (p.p.m.)* | |
|---|---|---|
| *Experiment*[1]*:* | *I* | *II* |
| Initial concentration[2] | 26–28 | 36–38 |
| After 5 dips[3] | 18–19 | – |
| After 20 dips | 11–12 | 18–20 |

[1] *Versuch – expériences*
[2] *Anfangskonzentration – concentration initiale*
[3] *Nach 5-maligem Eintauchen – après 5 trempages*

*Tabelle 1. Einfluss wiederholten Eintauchens auf die GA-Konzentration der Lösung*
*Tableau 1. Effet de trempages répétés sur la concentration GA dans la solution*

Table 2. Effect of repeated dipping on B9 concentration in the solution

| | *B9 concentration (g/l)* | |
|---|---|---|
| *Experiment*[1]*:* | *I* | *II* |
| Initial concentration[2] | 4.15 | 0.85 |
| After 6 dips[3] | 4.25 | 0.88 |

[1] *Versuch – expériences*
[2] *Anfangskonzentration – concentration initiale*
[3] *Nach 6-maligem Eintauchen – après 6 trempages*

*Tabelle 2. Einfluss wiederholten Eintauchens auf die B9-Konzentration der Lösung*
*Tableau 2. Effet de trempages répétés sur la concentration B9 dans la solution*

of B9 remains constant throughout repeated dips, but the concentration of GA gradually falls. This may be due to an active uptake by the tissue in addition to osmotic uptake, or to adsorption on starch grains which accumulate in the solution. Therefore, in contrast to the B9 solution which can be used many times, the GA solution must be renewed regularly. From a 1 p.p.m. GA solution and a 0.1 % B9 solution, respectively, about 0.05 μg GA and 50 μg B9 are absorbed by the parenchymatous tuber tissue of a single excised eye in 10 minutes. What proportion of this is actually involved in the breaking of dormancy and the growth of the bud is unknown.

### 3.2. The breaking of dormancy and subsequent growth

Generally, the GA method was found to equal or surpass the rindite-method in effect. Emergence is very even if the excised eyes are planted at a constant depth and the soil moisture and aeration are adequate.

Use of the GA method avoids the risk, inherent in the rindite-method, of discarding virus-infected tubers because they might tend to sprout less rapidly. Although there is no selection on the basis of sprouting with the GA method, percentage emergence is

Table 3. Emergence after rindite (RI) and GA treatment of excised eyes. Samples of 100 tubers per treatment; treated with rindite, or with 1 p.p.m. GA for 10 minutes.

| | % *of plants*[1] | | | | | | *Numbers of samples*[5] |
|---|---|---|---|---|---|---|---|
| | *normal*[2] | | *delayed*[3] | | *not emerged*[4] | | |
| | *RI* | *GA* | *RI* | *GA* | *RI* | *GA* | |
| Bintje | 94 | 91 | 3 | 8 | 3 | 1 | 24 |
| Irene | 95 | 96 | 2 | 2 | 3 | 2 | 10 |
| Furore | 95 | 93 | 3 | 0 | 2 | 7 | 9 |
| Ysselster | 98 | 97 | 1 | 1 | 1 | 2 | 5 |
| Surprise | 74 | 96 | 0 | 4 | 26 | 0 | 1 |
| Libertas | 66 | 64 | 14 | 16 | 20 | 20 | 1 |

[1] % *Pflanzen – % des plantes*
[2] *Normal – normales*
[3] *Verspätet – retardées*
[4] *Nicht aufgelaufen – non levées*
[5] *Anzahl Muster – nombre des échantillons*

*Tabelle 3. Auflaufen nach Rindite (RI)- und GA-Behandlung von ausgestochenen Augen. Muster von 100 Knollen pro Verfahren; behandelt mit Rindite oder mit 1 p.p.m. GA während 10 Minuten.*

*Tableau 3. Emergence d'yeux excisés après traitements aux RI et GA. Échantillons de 100 tubercules par traitement; traitement à la rindite ou au GA (1 p.p.m.) pendant 10 minutes.*

not noticeably lower, nor that of stragglers higher (Table 3). With varieties offering difficulties with rindite, the results with GA may be better, as was the case with *Surprise*, but not with *Libertas*, in this experiment.

Within varieties showing variable results with rindite, more homogeneous results have usually been obtained with GA, as is shown by data from 15 badly and well sprouted samples of the variety *Alpha* in Table 4. Varieties that usually respond well to rindite, respond equally well or better to GA.

Table 4. Emergence after treatment with GA of excised eyes from samples showing a variable response to rindite (RI). Subsamples of 100 tubers from 15 samples.

| *Sprouting with rindite*[1] | *Treatment*[2] | % *of plants*[3] | | |
|---|---|---|---|---|
| | | *normal*[4] | *delayed*[5] | *not emerged*[6] |
| Bad[7] | RI | 0 | 77 | 23 |
| | GA | 89 | 8 | 3 |
| Good[8] | RI | 87 | 9 | 4 |
| | GA | 80 | 16 | 4 |

[1] *Keimung nach Rindite-behandlung – germination avec rindite*
[2] *Verfahren – traitement*
[3] % *Pflanzen – % de plantes*
[4] *Normal – normales*
[5] *Verspätet – retardées*
[6] *Nicht aufgelaufen – non levées*
[7] *Schlecht – mauvaise*
[8] *Gut – bonne*

*Tabelle 4. Auflaufen nach Behandlung mit GA von ausgestochenen Augen aus Mustern mit unterschiedlicher Reaktion auf Rindite (RI). Teilmuster von 100 Knollen aus 15 Proben.*

*Tableau 4. Émergence après traitement au GA d'yeux excisés d'échantillons montrant une réaction variable à la rindite (RI). Parts de 100 tubercules provenant de 15 échantillons.*

Table 5. Rates of emergence and of growth after treatment with 1 and 5 p.p.m. GA. Samples of 20 tubers per treatment, immersed for 10 minutes.

| | *Number of plants emerged after*[1]: | | | | | | *Average plant height after 21 days*[2] *(cm)* | | *Regularity of stand (scale 1–5)*[3] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *6* | | *10* | | *14 (days)* | | | | | |
| *p.p.m. GA:* | *1* | *5* | *1* | *5* | *1* | *5* | *1* | *5* | *1* | *5* |
| Bintje | 2 | 7 | 20 | 19 | 20 | 19 | 16 | 19 | 3–4 | 3 |
| Sirtema | 4 | 11 | 20 | 19 | 20 | 19 | 13 | 15 | 3 | 2 |
| Furore | 0 | 6 | 20 | 20 | 20 | 20 | 10 | 17 | 4–5 | 4–5 |
| Alpha | 0 | 0 | 6 | 20 | 20 | 20 | 7 | 14 | 3 | 4 |
| Record | 0 | 8 | 17 | 20 | 20 | 20 | 9 | 21 | 4 | 4 |
| Burmania | 0 | 0 | 2 | 19 | 20 | 20 | 7 | 14 | 4 | 4 |
| Libertas | 0 | 0 | 7 | 18 | 19 | 18 | 6 | 10 | 2 | 4 |

[1] *Anzahl Pflanzen, aufgelaufen nach 6, 10 und 14 Tagen – nombre de plantes levées après 6, 10 et 14 jours*
[2] *Durchschnittliche Pflanzenhöhe nach 21 Tagen – hauteur moyenne des plantes après 21 jours*
[3] *Gleichmässigkeit der Entwicklung (Noten 1–5) – régularité du développement (échelle 1–5)*

*Tabelle 5. Auflauf- und Wachstumsraten nach Behandlung mit 1 und 5 p.p.m. GA. Muster von 20 Knollen pro Verfahren, eingetaucht während 10 Minuten.*

*Tableau 5. Vitesses d'émergence et de croissance après traitement avec 1 et 5 p.p.m. de GA. Échantillons de 20 tubercules par traitement, immersion pendant 10 minutes.*

Because of the linear uptake of the GA solution with time during the first few hours (Fig. 1), the measure of response depends on the product of exposure duration and concentration. Prolonged immersion may eventually cause damage due to anaerobiosis, but dips up to half an hour are harmless. Generally, a dip of 10 minutes in a 1 p.p.m. GA solution suffices to break dormancy completely without causing excessive stem elongation. The duration of the dip is not very critical, differences between 8 and 10 minutes being hardly appreciable. Rates of emergence and growth following dipping for 10 minutes in 1 and 5 p.p.m. GA are shown in Table 5. Emergence is completed sooner at the higher dosage, but final establishment is only improved in the case of a difficult variety such as *Libertas;* the sprouts of varieties which respond more easily often elongate too much and so become weak and liable to break off. In Table 6, 32 varieties are classified according to the length of their dormant period and rated on sturdiness of growth after the breaking of dormancy.

It should be noted that those varieties with a long dormant period always exhibit sturdy growth (although the reverse does not hold). Such varieties, often difficult to treat satisfactory with rindite, can be successfully treated with 5 p.p.m. GA and yet will not show excessive stem elongation. They may produce leaves with initially curled margins, as may also occur after rindite treatment, but this effect is temporary. Higher concentrations of GA cannot be recommended, since stem elongation occurs at the expense of leaf development and abnormal plant forms arise. At extreme concentrations such as 25 p.p.m. even thread-like sprouts occur.

Table 6. Sturdiness of growth from excised eyes of tubers of different varieties, classified according to the length of their dormant period.
1 = very weak, 2 = weak, 3 = moderate, 4 = sturdy.

| *Very short dormancy*[1] | | *Short dormancy*[2] | | *Moderate dormancy*[3] | | *Long dormancy*[4] | |
|---|---|---|---|---|---|---|---|
| Climax | 2 | Bintje | 2 | Ari | 3 | Alpha | 4 |
| Eigenheimer | 1 | Emergo | 3 | Béa | 3 | Ambassadeur | 4 |
| Furore | 3 | Ideaal | 3 | Eersteling | 2 | Arran Banner | 4 |
| Gineke | 3 | Irene | 3 | Patrones | 4 | Asoka | 4 |
| Sientje | 1 | Prefect | 2 | Pimpernel | 3 | Burmania | 4 |
| Urgenta | 4 | Record | 3 | Primura | 3 | Libertas | 4 |
| Voran | 1 | Sirtema | 2 | Spartaan | 3 | Maritta | 4 |
| | | IJsselster | 3 | | | Noordeling | 4 |
| | | | | | | Saskia | 4 |
| | | | | | | Surprise | 4 |

[1] *Sehr kurze Keimruhe – très courte dormance*
[2] *Kurze keimruhe – courte dormance*
[3] *Mittlere Keimruhe – dormance modérée*
[4] *Lange Keimruhe – longue dormance*

*Tabelle 6. Wuchskraft von Pflanzen aus ausgestochenen Augen von Knollen verschiedener Sorten in der Reihenfolge der Länge ihrer Ruheperiode.*
*1 = sehr schwach, 2 = schwach, 3 = mittel, 4 = kräftig.*

*Tableau 6. Vigeur de la croissance d'yeux excisés de tubercules de différentes variétés, classées selon la longueur de leur période de dormance.*
*1 = très faible, 2 = faible, 3 = modérée, 4 = vigoureux.*

The response of excised eyes to a GA treatment not only depends on the variety but also on the conditions under which the crop was grown and stored. Tubers grown during a predominantly warm summer usually have a shorter dormant period than tubers grown in a cool season (BURTON, 1963) and very young tubers usually have a shorter dormant period than the mature ones (SEMERDŽJAN and AVAKJAN, 1965). During prolonged storage, dormancy gradually fades (BURTON, 1963). The GA method is so flexible that it evens out such differences in the dormancy of samples of different varieties of various origins. In 1964, dormancy was not on the whole very pronounced and the GA concentration could be maintained at 1 p.p.m. In 1965 and 1966, on the other hand, considerable dormancy prevailed and the GA concentration had to be increased to 5 p.p.m. for varieties in which this condition was most marked.

The flexibility of GA treatment can be further increased by introducing B9 to reduce the effect of the GA on stem growth. B9 can be added to the GA solution or can be used in a second dip to save cost because of the necessity for regular renewal of the GA solution. It can also be sprayed on the plants after emergence and although some of the B9 falls on the soil, no after-effects on subsequent crops have been observed with soil in the green-house; with soil in pots there is no difficulty whatsoever (KRUG and BORCHARDT, 1966).

The application of B9 is only necessary when 1 p.p.m. GA alone is likely to induce excessive stem elongation as is the case when dormancy is over or nearly over. Varieties which produce sturdy sprouts after a long dormant period (Table 6) never need

B9, but the other varieties do, particularly when the growing or storage conditions have induced a short dormant period. In 1964, for instance, B9 was used on many samples, whereas it was hardly required in 1965 and 1966.

Very early harvested tubers are often barely dormant when treated and excellent results are then obtained with B9. In an experiment with *Bintje*, for example, samples were lifted on May 7, 14 and 21, and treated 0,1 and 2 weeks after lifting in the following ways: untreated, rindite, 1 p.p.m. GA, and 1 p.p.m. GA + 250 p.p.m. B9. The early harvested tubers did not stand the rindite treatment unless previously hardened by storage. With 1 p.p.m. GA, growth was initiated immediately, but without B9 the sprouts grew too long. With later liftings, rindite was tolerated better and treatment with GA alone gave good plants; the addition of B9 had hardly any effect.

It was generally found, that at the beginning of the testing season the use of B9 was profitable, but later on it was no longer effective or even resulted in an irregular stand. In an experiment, started on August 17, with 140 samples of the varieties *Alpha*, *Bintje* and *Sirtema*, a complete inspection four weeks after treatment was possible even where B9 had been used at 5000 p.p.m. On the other hand, in an experiment initiated on September 22, under very bad growing conditions, even 100 p.p.m. B9 considerably aggravated irregular emergence and poor growth (Table 7).

At the end of the storage period, however, when dormancy is practically over, B9 may again be useful to counteract the elongation effect of a GA treatment and to ensure a rapid and even emergence. Fig. 2 shows the reduction of stem growth by B9 in

Table 7. Effect of B9 treatment of excised eyes on emergence under poor growing conditions. Average values of 20 tubers from each of 19 varieties per treatment, dipped for 10 minutes in 1 p.p.m. GA and different concentrations of B9.

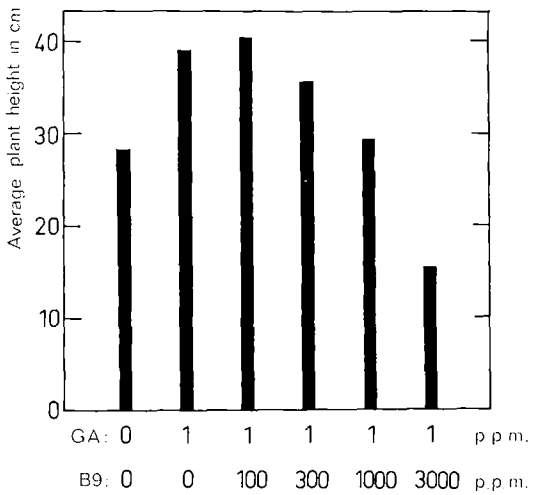
| *Concentration B9 (p.p.m.)* | *% of plants*[1] | | | *Average plant height*[5] *(cm)* | *Leaf development (scale 1–5)*[6] |
|---|---|---|---|---|---|
| | *normal*[2] | *delayed*[3] | *not emerged*[4] | | |
| 0 | 80 | 8 | 12 | 19 | 4.0 |
| 100 | 62 | 10 | 28 | 14 | 3.3 |
| 250 | 57 | 12 | 31 | 11 | 2.7 |
| 1,000 | 55 | 9 | 36 | 8 | 2.0 |
| 2,500 | 50 | 13 | 37 | 4 | 1.3 |

[1] *% Pflanzen – % de plantes*
[2] *Normal – normales*
[3] *Verspätet – retardées*
[4] *Nicht aufgelaufen – non levées*
[5] *Durchschnittliche Pflanzenhöhe – hauteur moyenne des plantes*
[6] *Blattentwicklung (Noten 1–5) – développement foliaire (échelle 1–5)*

*Tabelle 7. Einfluss der B9 Behandlung ausgestochener Augen auf das Auflaufen bei schlechten Wachstumsbedingungen. Mittlere Werte aus 20 Knollen jeder der 19 Sorten pro Verfahren, eingetaucht während 10 Minuten in 1 p.p.m. GA und verschiedene B9-Konzentrationen.*

*Tableau 7. Effet du traitement B9 d'yeux excisés sur la levée dans des conditions médiocres de croissance. Valeurs moyennes de 20 tubercules de chacune des 19 variétés par traitement, trempés pendant 10 minutes dans 1 p.p.m. GA et à différentes concentrations de B9.*

Fig. 2. Effect of B9 on plant height after breaking dormancy with GA. Tubers of *Bintje*, stored at at 2 C till January 27, then treated for 10 minutes and planted. Measurements on March 3.




Average plant height in cm – *durchschnittliche Pflanzenhöhe in cm – hauteur moyenne des plantes en cm*

*Abb. 2. Einfluss von B9 auf die Pflanzenhöhe nach Brechung der Keimruhe mit GA. Knollen der Sorte Bintje, bis 27. Januar bei 2 C gelagert, dann während 10 Minuten behandelt und ausgepflanzt. Messungen am 3. März.*

*Fig. 2. Effet de B9 sur la hauteur des plantes après rupture de la dormance avec GA. Tubercules de Bintje, conservés à 2 C jusqu' au 27 janvier, traités alors pendant 10 minutes et plantés. Mensurations le 3 mars.*

GA-treated excised eyes from tubers that still showed no sign of activity after prolonged storage at 2 °C, but nevertheless turned out to be no longer dormant upon transfer to 18 °C.

It can be concluded that B9 is required in those cases in which the tubers themselves contain insufficient growth inhibiting substances to balance the growth effect of the minimum amount of GA required to break dormancy. When these retarding substances are present in large quantities, as in those varieties with a long dormant period, the addition of B9 only causes or enhances irregular growth.

Generally, losses due to rotting of excised eyes are considerably less with the GA than with the rindite method. This holds good, particularly for excised eyes from early or freshly harvested tubers (see also ROGUSKI and MIERZVA, 1963) and from tubers from light soils. However, even with the GA method it was found desirable to destroy the foliage about a week prior to lifting. With samples in which some of the tubers are infected with *Phytophthora*, the apparently healthy ones can be selected when excising the eyes. In order to reduce losses still further, an antibiotic compound may be added to the GA solution. Since streptomycin and chloromycetin (chloroamphenicol) may cause chlorosis (BRUINSMA, 1965), the use of terramycin is to be preferred, at a con-

centration of 25 p.p.m. Our experience with this compound is however, very limited; terramycin cannot be combined with B9.

### 3.3. The assessment of virus symptoms

About 4–5 weeks after the GA treatment the plants can be inspected for virus symptoms and also used for serological and "A6" tests. With all the samples under investigation, the results were compared with those obtained with rindite-treated tubers; check samples known to be virus infected were also included.

One of the main objectives of the tests on tuber samples, following as they do the field inspection of the growing crops, is to detect late infections. Because of the greater possibility of multiplication and translocation of the virus in the tubers (MÜNSTER, 1965), the rindite method, in which the tubers remain intact for some weeks, might be more efficient for the detection of such late infections than the GA method in which the eyes are immediately excised. This, however, was not found to be the case in our experiments.

In one experiment, plants of the variety *Bintje* were artificially infected with virus $Y^n$ in the field, 4 or 3 weeks before destruction of the foliage and samples of their tubers were treated with rindite or with GA. The results (Table 8) demonstrate that the two methods are equally sensitive and produce comparable figures for late-infected tubers.

Another experiment was carried out with 175 samples of the variety *Doré*, which is

Table 8. Detection of late virus infections by means of the GA and rindite methods, respectively. (*Bintje* plants infected with virus $Y^n$ in the field 4 and 3 weeks prior to destruction of the foliage in preparation for lifting and treatment 1 week later).

| *Time of infection*[2]: | *Number of cuttings*[1] | | | | | | |
|---|---|---|---|---|---|---|---|
| | *4 weeks* | | | *3 weeks* | | *not infected*[3] | |
| | *emerged*[4] | *diseased*[5] | % | *emerged*[4] | *diseased*[5] | *emerged*[4] | *diseased*[5] |
| 1 p.p.m. GA | 23 | 15 | 65 | 24 | 0 | 19 | 0 |
| 1 p.p.m. GA + 0.01 % B9 | 24 | 20 | 83 | 22 | 0 | 24 | 0 |
| 1 p.p.m. GA + 0.1 % B9 | 25 | 19 | 76 | 23 | 0 | 28 | 0 |
| rindite | 20 | 13 | 65 | 21 | 0 | – | – |

[1] *Anzahl Stecklinge – nombre de boutures*
[2] *Zeitpunkt der Infektion (Wochen) – époque de l'infection (semaines)*
[3] *Nicht infiziert – non infectées*
[4] *Aufgelaufen – levées*
[5] *Krank – malades*

*Tabelle 8. Feststellung später Virusinfektionen mittels der GA- und der Rinditemethode. (Im Felde – 4 und 3 Wochen vor der Krautvernichtung als Erntevorbereitung – mit Virus $Y^n$ infizierte Pflanzen der Sorte Bintje und Behandlung eine Woche nach der Ernte.)*

*Tableau 8. Détection d'infections virologiques tardives par les méthodes GA et rindite respectivement. (Plantes Bintje infectées avec le virus $Y^n$ dans le champ 4 et 3 semaines avant la destruction du feuillage en vue de l'arrachage et traitement 1 semaine plus tard.)*

Table 9. Assessment of infection with viruses $Y^n$ and A, visually and by the "A6"-test, on plants from tubers treated with rindite or excised eyes treated with GA and B9 (var. *Doré*)

| *Virus* | *Test* | *Total number of diseased plants in 175 samples of*[1]*:* | | | |
|---|---|---|---|---|---|
| | | *25 tubers* 5 *p.p.m. GA* + 0.25 % *B9* | *25 tubers* 5 *p.p.m. GA* + 0.5 % *B9* | *50 tubers GA* + *B9* | *50 tubers rindite* |
| $Y^n$ | "A6" | 19 | 32 | 51 | 42 |
| | visual[2] | 16 (84 %) | 16 (50 %) | 32 (63 %) | 31 (74 %) |
| A | "A6" | 23 | 24 | 47 | 16 |
| | visual[2] | 10 (43 %) | 2 ( 8 %) | 12 (26 %) | 4 (25 %) |

[1] *Gesamtzahl kranker Pflanzen in 175 Mustern von 25 und 50 Knollen – nombre total de plantes malades dans 175 échantillons de 25 et 50 tubercules*
[2] *Visuell – visuel*

*Tabelle 9. Beurteilung der Infektion mit den Viren $Y^n$ und A (visuell und A im A6-Test) bei Pflanzen aus rinditebehandelten Knollen oder mit GA- und B9-behandelten Augenstücken (Sorte Doré)*

*Tableau 9. Détermination de l'infection par les virus $Y^n$ et A, visuellement et par test A6, sur des plantes provenant de tubercules traités à la rindite ou d'yeux excisés traités au GA et B9 (var. Doré)*

extremely difficult to inspect visually. A comparison of the results (Table 9) shows again that the GA method was not inferior to the rindite method. On the contrary, the larger numbers of infected plants detected in the "A6"-test following GA treatment than following rindite treatment, shows that the danger of discarding the poorer sprouting infected tubers in the rindite method is greater than the danger of reducing the amount of infection detected by the immediate excision of the eyes in the GA method. From a comparison of the percentages of plants found to be infected in the "A6"-test and which were also visually detected, it appears that in this difficult variety the visual inspection of GA-treated material is no more and no less efficient than it is with plants from rindite-treated tubers. However, high concentrations of B9 were used in this early experiment and it can be seen that lowering the concentration from 0.5 to 0.25 % appreciably increased percentage detection. The reason for this is that the green colour of the leaves of plants grown from excised eyes treated with B9 is darker the higher the concentration (see also KRUG and WIGGER, 1964) and that the dark green colour tends to mask discoloration caused by the virus. At the lower B9 concentrations this tendency decreases and plants from cuttings treated with GA alone often look slightly paler (BRUINSMA, 1965) which facilitates the assessment of mosaic patterns.

Mosaic symptoms are sometimes obscured with the rindite method also, owing to initial variegation and distortion of the leaflets, e.g. in the varieties *Sirtema* and *Red Pontiac*.

For these reasons, inspectors with long experience of visual assessment of virus symptoms in plants grown from excised eyes definitely prefer plants grown from GA-treated tubers.

## 4. DISCUSSION

In order to replace the breaking of dormancy with rindite by another chemical treatment, various substances, alone and in combinations, were tried out, e.g. gibberellic acid, ammonium thiocyanate and α-tocopherol (BRUINSMA, 1963).

Of these compounds only GA turned out to be promising. Of the two growth retardants tested, B9 was the most satisfactory, 2-chloroethyl-trimethylammonium chloride (CCC) was found to have more side effects on leaf form and colour, as previously described by KRUG and WIGGER (1964). Therefore, the further development of the method was confined to GA and B9.

Most probably, GA acts on the dormancy of the potato bud in a similar way as does ethylene chlorohydrin, the main component of rindite, i.e. by activating the synthesis of nucleic acids in the cell nucleus (TUAN and BONNER, 1964; BRUINSMA, 1966). Gibberellin-like substances are, moreover, natural components of the potato plant (HAYASHI and RAPPAPORT, 1962, 1965; BRUINSMA, 1962; WHEELER, 1962; WHEELER and HUMPHRIES, 1963) and their content increases at sprouting (SMITH and RAPPAPORT, 1960). B9 is involved in the inhibition of the biosynthesis of naturel growth-regulating substances (REED, MOORE and ANDERSON, 1965) and is required only when the endogenous inhibitor content is low, e.g. with very young and very old tubers of weak-growing varieties.

The ability of GA to break the dormancy of potato buds is well-known (BLUMENTHAL-GOLDSCHMIDT and RAPPAPORT, 1965; BRUINSMA, 1962; EMILSSON and LINDBLOM, 1963; LIPPERT, RAPPAPORT and TIMM, 1958; TIZIO, 1964). Even earlier LUDWIG (1958) applied it as a growth stimulator to overcome the poor growth of excised eyes, but because this was done after storage, real dormancy was probably no longer involved. For similar reasons, GA has recently been sprayed on emerged shoots growing from excised eyes by KRUG and BORCHARDT (1966). NEČAS (1965) reported on the use of gibberellin, alone and in combination with rindite, to stimulate sprouting and growth from excised eyes. He applied drops of 50 p.p.m. gibberellin ($A_1$:$A_3$ = 3:2) directly to the eyes.

We found dipping of excised eyes in GA solutions to be a convenient and rapid technique suitable for use with large numbers of samples as a routin treatment. The low concentration, usually 1 p.p.m. and up to 5 p.p.m. at most, keeps the cost down notwithstanding the regular renewals necessary because of the gradual dilution of the solution. The more expensive B9 solution, which, however, does not become diluted during repeated dips, can be used separately by transferring samples from one solution to the other without altering the rate of absorption of the solution. Draining for a few minutes before transfer is recommended to avoid excessive contamination of the second dipping bath. Handling is greatly facilitated by keeping the excised eyes of a sample together in a large-mesh bag. A dipping time of 10 minutes in each solution was always found to be sufficient.

Although for the present purpose the use of excised eyes is quite satisfactory, it is to be regretted that the method cannot be applied to whole tubers for such other aims

as the growing of a second crop or the mechanical planting of unsprouted tubers. Our results in this respect agree with the negative ones obtained by VAN HIELE (1961).

Even damaging the tuber surface with nails (SLOMNYCKI and RYLSKI, 1964) did not allow of a safe adaption of the method for these other practical objectives. Possibly potato seed cutting machines might be useful for these purposes.

The treatment of sprouted tubers with GA, in order to ensure a rapid and even emergence, involves the risk of extreme elongation of the already initiated stolons and of a delay of tuberisation that may cause a considerable reduction in yield.

In conclusion, the GA method has the following advantages over that using rindite.

1. Duplicate sampling is unnecessary since the heel ends of the same tubers, after excision of the apical eyes, can be used in the Igel-Lange test.
2. Samples can be treated immediately without previous storage. In contrast to rindite, GA is also tolerated by young tubers.
3. Rotting does not follow mechanical damage.
4. With the excision of the eye-pieces, no particular care because of sprouted eyes has to be taken.
5. In contrast to rindite, GA is non-toxic and has no unpleasant smell.
6. The GA method is safer and more flexible, and the margin between effective and toxic doses is much wider. The excessive stem elongation, curling of leaf margins, and leaf chlorosis, which follow dipping in too high GA concentrations, can be counteracted by treatment with the equally non-toxic growth retardant B9, in solutions of 100 – 5,000 p.p.m. It is particularly this ability to balance the effect of GA with B9 that makes the method so flexible and adaptable for use with different varieties and under different conditions.
7. Inspection for the various virus symptoms is generally easier with plants from GA-treated than those from rindite-treated material, because the leaves are usually more evenly coloured and flatter. However, treatment with B9 tends to lessen this advantage owing mainly to its darkening effect on leaf colour.
8. Because the interval between the planting and the inspection is about equal with the two methods, the replacement of gasing and pre-sprouting by a 10 minute dip results in a considerable saving of time. This is especially important at the beginning of the inspection period, when the storage of tubers can also be omitted, and at the end, in autumn, when natural growing conditions rapidly get worse.
9. Samples containing rotting tubers may deteriorate completely during the pre-sprouting period following the rindite treatment. With the GA treatment, apparently healthy tubers can be selected for the excision of eyes and rotting may be limited still further by the addition of an antibiotic substance, such as terramycin, to the dipping solution. Infection of samples by dipping together with, or after diseased samples, was never found to occur.
10. The theoretical danger, that the early excision of eyes might prevent their infection by virus that is being multiplied and translocated in a newly infected tuber, has been shown to be of no more importance than the risk inherent to the rindite

method of discarding infected tubers because of delayed sprouting when the eyes are excised at the end of the pre-sprouting period.
For these reasons we recommend the GA method for the breaking of dormancy of potato buds and for regulating the growth of the subsequent plants.


## ZUSAMMENFASSUNG

### ÜBER DIE ANWENDUNG VON GIBBERELLINSÄURE (GA) UND N-DIMETHYLAMINOBERNSTEINAMINSÄURE (B9) BEIM NACHWEIS VON VIRUSKRANKHEITEN IN SAATKARTOFFELN

Zur Unterbrechung der Keimruhe und zur Wachstumsregulation der Augenstecklinge wurde eine Methode entwickelt, die gegenüber der Rinditemethode bedeutende Vorteile aufweist. Die Endknospe mit einem Stück Knollengewebe wird ausgeschnitten, 10 Minuten in eine GA-Lösung getaucht und ausgepflanzt. In der Eintauchzeit absorbieren die Knollenstücke etwa 1 % ihres Frischgewichtes (Abb. 1). Die sich bei diesem Verfahren entwickelnden Stecklinge können fast zu gleicher Zeit beurteilt werden wie gleichzeitig ausgepflanzte vorgekeimte Augen nach Rinditebehandlung, obwohl nicht auf Auskeimen der Augen selektiert wurde (Tabelle 3). Sorten, die mit Rindite nur schwer zum Auskeimen gebracht worden können, reagieren oft gut auf GA (Tabelle 4).
Zur Brechung der Keimruhe genügt in der Regel eine Konzentration von 1 p.p.m. GA; für Sorten mit ausgeprägter Keimruhe kann sie bis 5 p.p.m. erhöht werden (Tabelle 5). Wenn nur eine sehr kurze Keimruhe zu erwarten ist, z.B. bei bestimmten Sorten (Tabelle 6) und bei sehr jungen oder sehr alten Knollen, können die Knollenstücke nach der GA-Behandlung in eine B9-Lösung (100-5000 p.p.m.) getaucht werden, um abnormales Stengelwachstum zu verhindern (Abb. 2). Unter ungünstigen Wachstumsverhältnissen kann aber B9 unregelmässiges Auflaufen noch verschlimmern (Tabelle 7). Zudem werden die Blätter dunkler grün, wodurch die Beurteilung von Virussymptomen erschwert wird (Tabelle 9). Die mit GA behandelten Stecklinge sind übrigens in der Regel leichter zu beurteilen als die mit Rindite behandelten.
Bei Spätinfektionen ergeben beide Methoden den gleichen Befund (Tabelle 8).
Die GA-Methode wird gegenüber der Rindite-Methode namentlich deshalb bevorzugt, weil sie eine doppelte Probenentnahme für den Igel-Lange-Test überflüssig macht. Daneben ist sie rasch, bequem, leicht an bestimmte Proben anzupassen und nicht giftig. Eine Aufbewahrung von Knollen ist nicht nötig, und Fäulnis ist leichter zu vermeiden. Die Methode erweist sich als besonders vorteilhaft bei der Verarbeitung vieler Proben verschiedener Herkunft.



## RÉSUMÉ

### L'UTILISATION DE L'ACIDE GIBBERELLIQUE (GA) ET DE L'ACIDE N-DIMETHYLAMINOSUCCINAMIQUE (B9) DANS LE TEST DE BOUTURES D'YEUX

Les auteurs ont développé une méthode pour rompre le repos végétatif et régulariser la végétation des boutures d'yeux, qui présente un avantage significatif sur la méthode à la rindite. Le bourgeon terminal est excisé avec un fragment de tissu du tubercule, trempé 10 minutes dans une solution GA et planté. Pendant ce temps, les fragments absorbent quelque 1 % de leur poids frais (Fig. 1). Les boutures sont presque en même temps à examiner que les yeux prégermés transplantés aussitôt après traitement à la rindite, bien qu'il ne soit fait aucune sélection de

germes. Les variétés qui germent difficilement avec la rindite réagissent bien au GA (Tableau 4). La plupart du temps, il suffit de 1 p.p.m. GA, les yeux de repos plus profond pouvant recevoir jusque 5 p.p.m. (Tableau 5). Quand on s'attend à un très faible repos végétatif, par ex. avec les variétés mentionnées au Tableau 6, et lorsque les tubercules sont très jeunes ou très vieux, on peut, le plus près du traitement au GA, immerger dans 100–5000 p.p.m. B9 pour supprimer le développement anormal de tiges (Fig. 2). Sous des conditions défavorables de croissance B9 peut cependant aggraver un développement irrégulier (Tableau 7). De même le feuillage est d'un vert plus foncé, de sorte que la détermination des symptômes de viroses est plus difficile (Tableau 9). Au demeurant les plantes traitées au GA étaient le plus souvent plus faciles à examiner que celles traitées à la rindite.

Dans le cas d'infections tardives les deux méthodes donnent les mêmes résultats (Tableau 8). La méthode GA est surtout préférée à la méthode rindite, attendu qu'elle rend superflu un nouvel échantillonnage pour le test Igel-Lange. En outre, elle est rapide, aisée, facile à adapter à un échantillon déterminé, et non toxique. Un stockage des tubercules est inutile et la pourriture est plus facile à éviter. Elle se montre particulièrement avantageuse pour la mise en oeuvre d'échantillons de plus gros volume de diverses origines.

## REFERENCES


ARENZ, B. und HUNNIUS, W. (1963): Erfahrungen mit dem Igel-Lange-Test in der Serienarbeit. *Bayer. landw. Jb.* **40**, 122–136.

BAKKER, D., BRUINSMA, J. en SINNEMA, A. (1966): Kiemrustbreking met gibberelline van aardappelknollen, bestemd voor nakontrole. *Meded. ned. alg. KeurDienst LandbZaden Aardappelpootg.* **22**, 94–98.

BLUMENTHAL-GOLDSCHMIDT, S. and RAPPAPORT, L. (1965): Regulation of bud rest in tubers of potato *Solanum tuberosum* L. II. Inhibition of sprouting by inhibitor β-complex and reversal by gibberellin $A_3$. *Pl. Cell Physiol., Tokyo* **6**, 601–608.

BOKX, J. A. DE (1964): Onderzoekingen over het aantonen van aardappel-$Y^N$-virus met behulp van toetsplanten. Thesis, Wageningen, 84 pp.

BRUINSMA, J. (1962): A survey of recent Japanese research on dormancy in potato tubers. *Eur. Potato J.* **5**, 195–203.

BRUINSMA, J. (1963): α-Tocopherol (vitamin E) as a plant growth regulator. *Chem. Weekbl.* **59**, 599.

BRUINSMA, J. (1965): Effects of pesticidal treatments on the chlorophyll content of plant parts. *Residue Rev.* **10**, 1–39.

BRUINSMA, J. (1966): Plant growth regulators: toys and tools. *Meded. Rijksfac. Landbouwk. Gent* **31**, 343–369.

BURTON, W. G. (1963): Concepts and mechanism of dormancy. In: The growth of the potato. Butterworth, London, pp. 17–41.

CHOUDHURI, H. and GHOSE, S. (1963): Effect of gibberellic acid on sprouting, growth of internodes, tuber shape and yield in different varieties of potatoes. *Eur. Potato J.* **6**, 160–167.

EMILSSON, B. and LINDBLOM, H. (1963): Physiological mechanisms concerned in sprout growth. In: The growth of the potato. Butterworth, London, pp. 45–62.

GRECHUSHNIKOV, A. I., KIRYUKHIN, V. P., SEREBRENIKO, V.S. and TEKTONIDI, I. P. (1964): Some physiological and biochemical changes in potato produced by treating the tubers with gibberellin. *Soviet Pl. Physiol.* **11**, 530–537.

HAYASHI, F. and RAPPAPORT, L. (1962): Gibberellin-like activity of neutral and acidic substances in the potato tuber. *Nature, Lond.* **195**, 617–618.

HAYASHI, F. and RAPPAPORT, L. (1965): In vitro conversion of neutral gibberellin-like substances from potato tubers. *Nature, Lond.* **205**, 414–415.

HIELE, F. J. H. VAN (1961): Unsprouted potato tubers with gibberellic acid ($GA_3$). *Eur. Potato J.* **4**, 26–39.

KELLER, E. R. and BERCES, S. (1966): Check-testing for virus Y and leaf-roll in seed potatoes with particular reference to methods of increasing precision with the A6-leaf test for virus Y. *Eur. Potato J.* **9**, 1–14.

KÖHLER, E. (1953): Der *Solanum demissum*-Bastard "A6" als Testpflanze verschiedener Mosaikviren. *Züchter* **23**, 173–176.

KRUG, H. und BORCHARDT, G. (1966): Wachstumskontrolle von Kartoffel-Augenstecklingen. *Kartoffelbau* **17**, 304–306.

KRUG, H. und WIGGER, E. A. (1964): Neuer Wirkstoff als Wachstumsregulator für Augenstecklinge. *Kartoffelbau* **15**, 276–278.

LADYGINA, E. A. (1964): Einfluss der Wuchsstoffe auf die Veränderung der physiologischen Vorgänge bei Kartoffelpflanzen. *Trudy naučno-issled. Inst. Kartof. Hozjajstva* **3**, 16–24.

LIPPERT, L. F., RAPPAPORT, L. and TIMM, H. (1958): Systemic induction of sprouting in white potatoes by foliar application of gibberellin. *Pl. Physiol., Wash.* **33**, 132–133.

LUDWIG, A. (1958): Die Verwendung von Gibberellin bei der Augenstecklingsprüfung von Pflanzkartoffeln. *Z. landw. Vers.- u. UntersWes.* **4**, 387–401.

MÜNSTER, J. (1965): Hinweise zu den Igel-Lange- und *Solanum* A6-Testen im Rahmen der Saatkartoffelanerkennung. *Kartoffelbau* **16**, 215–218.

NEČAS, J. (1965): Die Anwendung des Gibberellins bei der Augenstecklingsprüfung des Kartoffelpflanzgutes. *Rostlinná Vijroba (Sb. čsl. Akad. zeměd. Věd, Rada C)* **8**, 195–200.

PODOJIL, M. and ŠEVÍČK, V. (1960): Quantitative estimation of gibberellic acid by paper chromatography. *Folia microbiol.* **5**, 192–197.

REED, D. J., MOORE, T. C. and ANDERSON, J. D. (1965): Plant growth retardant B-995: a possible mode of action. *Science, N.Y.* **148**, 1469–1471.

ROGUSKI, K. und MIERZVA, Z. (1963): Studium der Verfahren zum früheren erwecken der Knollen aus der Keimruhe. *Biul. Inst. Hodowli i Aklimat. Roślin* **3**, 77–82.

SEMERDŽJAN, S. P. und AVAKJAN, C.M. (1965): Unterbrechung der Keimruhe frischgeernteter Kartoffelknollen mit Gibberellin. *Fiziologiya Rast.* **12**, 164–166.

SLOMNYCKI, I. and RYLSKI, I. (1964): Effect of cutting and gibberellin treatment on autumn-grown seed potatoes for spring planting. *Eur. Potato J.* **7**, 184–192.

SMITH, O. E. and RAPPAPORT, L. (1960): Endogenous gibberellins in resting and sprouting potatoes. *Adv. Chem. Ser.* **28**, 42–48.

SMITH, O. E. and RAPPAPORT, L. (1965): Sprouting, plant growth, and tuber production as affected by chemical treatment of seed pieces. V. Respiration and sprouting of gibberellin $A_3$-treated white potatoes held at several storage temperatures. *Am. Potato J.* **42**, 165–173.

TIZIO, R. (1964): Cycle végétatif, et mécanisme de tubérisation chez la pomme de terre. *Pomme Terre fr.* **26**, (301), 3–20.

TUAN, D. Y. H. and BONNER, J. (1964): Dormancy associated with repression of genetic activity. *Pl. Physiol., Wash.* **39**, 768–772.

WHEELER, A. W. (1962): Growth activity of the gibberellins of dwarf french bean, potato and lettuce. *J. exp. Bot.* **13**, 36–44.

WHEELER, A. W. and HUMPHRIES, E. C. (1963): Effect of gibberellic acid on growth, gibberellin content and chlorophyll content of leaves of potato (*Solanum tuberosum*). *J. exp. Bot.* **14**, 132–136.