Potato Research 28 (1985) 361–377

Investigation of sprout-growth-inhibitory compounds in the volatile fraction of potato tubers

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Accepted for publication: 20 December 1984

Zusammenfassung, Résumé p. 375

Additional keywords: storage, cv. Désirée, trapping of volatiles, gas-chromatographic separation, diphenylamine, dibenzothiophene

Summary

The fractionation of volatile substances produced by potato tubers using gas liquid chromatography (GC) was followed by assaying fractions for sprout-growth-inhibitory activity using a potato shoot-tip bioassay. A region of the chromatogram having high sprout-growth-inhibiting activity was identified and subsequently further resolved by capillary column GC into several peaks, 5 of which gave well-defined mass spectra. Two of these compounds were identified as diphenylamine and dibenzothiophene. Diphenylamine showed high growth-inhibitory activity in the bioassay and was shown to be an effective sprout suppressant for whole tubers. The compound was tested in small-scale storage trials using up to 0.5 tonne of potatoes to assess its potential as a sprout suppressant.

Introduction

Burton (1952a, b) observed that stored potato tubers produce volatile compounds that can inhibit the growth of potato sprouts. He was able to rule out both respiratory CO_2 and ethylene as the active principle inhibiting sprouting in tubers under storage conditions in which the volatile products of the potato were allowed to accumulate (Burton, 1952, Burton & Meigh, 1971). A systematic investigation of the problem was made by Meigh et al. (1973), who extracted potato peel samples with ether and separated components of the ether-soluble fraction by gas liquid chromatography (GC). They identified several of the major constituents of this fraction by combined gas chromatography/mass spectrometry (GC/MS). Among the compounds found were a group of methylated naphthalenes which were subsequently shown to inhibit sprout growth. In this group 1,4- and 1,6-dimethylnaphthalene were the most active sprout suppressants. Meigh et al. (1973) however, concluded that the activity in the total potato volatile fraction could not be explained in terms of the activity due to naphthalenes alone and that it was thus likely that other, possibly more active compounds might be present.

In re-examining this problem, we have taken a somewhat different approach. This involved the need for a sensitive bioassay which could be applied to the small quantities of volatile compounds obtained from intact tubers (Burton & Meigh, 1971). Recently Filmer & Thompson (1983) have developed a bioassay based on the growth

of sterile excised potato shoot-tips, which is sensitive to a range of volatile growth-inhibitors. This method has been used in the present work to show the sprout-growth-inhibitory activity of the volatile substances evolved from potato tubers. We have followed the distribution of this activity between various fractions separated by GC. Only when the biological activity could be localized to a relatively narrow region of the chromatograph, was the chemical nature of the compounds present investigated. This approach has led to the identification of a novel biologically active compound which has subsequently been evaluated as a potential sprout suppressant.

Materials and methods

Collection of potato volatiles

One tonne of washed potato tubers, cv. Désirée, was stored at 10 °C in a sealed galvanized tank through which air, filtered through charcoal, was drawn continuously at 400 l/hr. The effluent air from the tank was passed through a glass vessel held in a refrigerated bath at −90 °C. Frozen aqueous condensate containing the potato volatiles collected in the trap which was replaced at weekly intervals. The condensate was allowed to thaw while in contact with an equal volume of ether and it was extracted twice with equal volumes of ether. The ether phase was evaporated in vacuo to yield a small quantity of oily residue which was subsequently redissolved in ethanol. In practice, the residues from the collection of condensates from four successive 7-day periods were combined and made to a total of 2 ml with ethanol. This was designated the total volatile fraction. Control samples, used to assess the extent of chemical contamination of the potato volatile fraction by environmental pollutents such as hydrocarbons and phthalate plasticizers, were collected in the same way after passing air through an empty galvanized storage tank. This condensate was extracted with ether and analysed by GC for contaminants.

Separation of compounds by gas chromatography and the investigation of their identity by mass spectroscopy

In the initial GC separations, from which fractions were tested in the bioassay for sprout-inhibitory activity, a packed column of Carbowax 20M was used, but at later stages, when improved resolution was required for combined GC/MS studies, a capillary column with the same stationary phase was used. The conditions of chromatography were:

– *packed-bed column* 1.5 m×4 mm 3 % Carbowax 20M on 120–140 mesh CQ (Jones Chromatography Ltd, Llanbradach, Wales); temperature programme 100–200 °C at 4 °C/min; carrier gas argon at 45 ml/min; Flame Ionisation Detector (FID).

– *capillary column* 25 m×0.235 mm Carbowax 20M (Quadrex Corp., New Haven, Conn., USA); temperature programme 100–200 °C at 4 °C/min; carrier gas helium at 124 kN/m^2; detector FID.

Fractions of the eluant from the packed bed column were trapped in V-shaped capillary traps (1 mm×15 cms) cooled in solid CO_2. A flow splitter (March, 1979) was used which allowed 10 % of the flow to go to the detector while the rest was collected in the trap. Fractions containing compounds eluting within different temperature ranges were obtained by replacing traps at appropriate points in the temperature gradient. The position of the cuts between fractions was determined in relation to

the elution position of known compounds.

Fractions collected in the capillary traps were recovered by washing the traps with 500 μl ethanol which was then used either for test in the bioassay or for re-chromatography.

The fraction from the packed column showing most activity in the bioassay was applied to the capillary column and this gave much improved resolution, but the levels of compounds applied was so low that re-collection and bioassay of the compounds thereafter was not feasible. The capillary column was connected to the MS by inserting the column directly into the capillary glass interface as far as the pressure-reducing activator, sealing against the atmosphere with a Graphlok coupling (SGE (UK) Ltd., London NW2 7AY).

Where MS data gave clear information on the identity of the unknown compound, samples of the pure chemical were obtained and chromatogrammed under the standard conditions and their identity confirmed by comparing the mass spectra and retention times of the unknown and known samples.

Bioassay of potato volatile compounds

The shoot-tip bioassay of Filmer & Thompson (1983) was used to identify the sprout-growth-inhibitory activity of the volatile fractions before and after separation by GC. Samples of the ethanolic solutions containing the volatile compounds were applied to glass fibre strips and placed in culture tubes in which the shoot-tips, one per tube, were grown (Filmer & Thompson, 1983) over a 7–10 day period at 25 °C in the dark. Each sample was analysed in at least 10 and usually 20 replicate cultures. Results are expressed as percentage inhibition of growth in the treated cultures compared with the control. Known compounds were applied over a range of concentrations and the results are expressed as the percentage growth of the treated shoot-tips compared to a control.

Further tests for sprout-suppressant activity

Compounds in the active fraction identified by MS were initially tested for sprout-inhibitory activity in the shoot-tip bioassay. One compound showing significant activity, diphenylamine (DPA), was further investigated in a range of assays based on whole tubers. Owing to the relatively low volatility of DPA, the whole-tuber bioassay of Meigh (1969) was unsuitable. A whole-tuber bioassay was developed in which 2-kg samples of tubers were either sprayed or painted with the compound dissolved in acetone. They were then stored in glass vessels through which air at 10 °C was passed at 80 ml/min for up to 4 weeks. Control tubers were treated with acetone alone.

A further series of small-scale storage trials with 500-kg batches of potatoes were carried out under the conditions described by Filmer & Rhodes (1983). Two methods of dispersion of the compound were tested. In one, DPA was dissolved in acetone and applied as a fog at the base of the chamber, a method successfully used by Filmer & Rhodes (1983) to test 1,4,6-trimethylnaphthalene. A second method was used in a later experiment in which DPA was mixed with alumina (40 g DPA/kg alumina). The resultant powder was dusted onto the tubers at 2.5 g/kg tubers to give an applied DPA concentration of 100 mg/kg. All tubers were stored at 10 °C. At intervals, samples were taken for measurement of sprout growth and residual levels of DPA. For the analysis of DPA, potatoes from the storage cabinets were peeled and samples of peel (1–2 mm deep) were frozen in liquid nitrogen. Weighed subsamples were ex-

tracted overnight with dichloromethane in a Soxhlet extractor; extract was taken to dryness and the residue dissolved in ethanol and analysed for DPA by GC. The GC conditions were: *column* 1.5 m×4 mm 3 % Apiezon L (Apiezon Products Ltd., London) on 120–140 mesh CQ (Jones Chromatography Ltd); oven temperature 180 °C; detector temperature 250 °C; argon carrier gas at 45 ml/min.

The output from the FID detector was logged on a mini-computer (DEC PDP 11/34) and the integrated areas under the peaks compared with that given by samples of DPA of known concentration. The recovery of DPA, under the conditions described, is 84–85 %, which compares well with other published methods (Allen & Hall, 1980).

Results

Table 1 shows the activity of the total potato volatile fraction in the shoot-tip bioassay. About 10 µl of the fraction gave 50 % inhibition of growth in the bioassay. Fig. 1 shows the separation of the total volatile fraction by GC on a packed column of Carbowax 20M. Also shown are the elution positions of sprout suppressants found in previous work (Meigh et al., 1973). Initially the mixture of volatiles was resolved into 3 fractions (F1–F3, Fig. 1) representing different segments of the temperature programmed runs. Their biological activity was tested in the shoot-tip bioassay (Table 2) and sprout-growth-inhibitory activity was present mainly in fraction 3. Fraction 3 was further resolved into 3 subfractions (F3a–F3c, Fig. 1) representing regions of

Table 1. Growth-inhibitory activity of the total volatile fraction (TVF) in the shoot-tip bioassay.

Sample [1]	Average increase of shoot length per day (mm ± s.e.m.) [2]	Inhibition (% of control) [3]
Control [4]	0.90 ± 0.3	0
10 µl* TVF	0.45 ± 0.1	56
25 µl* TVF	0.34 ± 0.07	62
50 µl* TVF	0.19 ± 0.05	83

* Aliquot from a TVF solution added to each culture tube – *Die in jedes Kulturröhrchen zugegebene aliquote Menge aus einer TVF-Lösung – Partie aliquote de la TVF ajoutée à chaque tube de culture.*

[1] *Probe – Echantillon;* [2] *Mittlere Zunahme der Sprosslänge pro Tag (mm ± Mittlere Standardabweichung) – Augmentation moyenne de la longueur des pousses par jour (mm ± erreur standard de la moyenne);* [3] *Hemmung (% der Kontrolle) – % d'inhibition (% du témoin);* [4] *Kontrolle – Témoin*

Tabelle 1. Wachstumshemmende Wirkung der gesamten flüchtigen Fraktion (TVF) im Triebspitzen-Biotest.
Tableau 1. Activité inhibitrice de la croissance de la fraction volatile totale (TVF) dans un essai biologique sur extrémités de pousses.

the chromatogram eluting between 180–200 °C and during isothermal elution at 200 °C. Analysis of these fractions in the bioassay (Table 2) suggests that the activity is concentrated in the region between 180–200 °C and during the early stages of isothermal elution at 200 °C.

Fig. 2 shows the improved resolution of the combined fractions 3a and 3b (Fig. 1) on a capillary column of Carbowax 20M. This region was resolved into 14 peaks some of which gave well-defined mass spectra. One, peak 5 (retention time 27.7 min), gave an intense mass peak at m/e 169 and peaks of lower intensity at m/e 168, 167, 170, 51, 77. Fig. 3 shows the comparison of the mass spectrum of authentic DPA (A) with that obtained for peak 5 (B). Further, DPA was shown to co-chromatogram with peak 5, and this confirmed its identity. Similarly, peak 7 (retention time 28.2 min) with one intense mass peak at m/e 184 was identified as dibenzothiophene (DBT) by comparison with the authentic compound.

Three further peaks (6, 11 and 12; Fig. 2) gave mass spectra with main ions of the following masses: peak 6 (retention time 28 min) m/e 86, 55, 183, 98, 56; peak 11 (retention time 29.6 min) m/e 184, 77, 141, 51, 227; peak 12 (retention time 29.7 min)

Table 2. Growth-inhibitory activity of volatiles in the shoot-tip bioassay following fractionation of the total volatile fraction on Carbowax 20M by gas chromatography.

Fraction No [1]	Temperature range [a,2] (°C)	Growth rate (mm/day) [3]	Inhibition [4] (%)
Control [5]		1.13 ± 0.13	
F1	120 – 150	1.25 ± 0.15	0
F2	150 – 180	1.42 ± 0.19	0
F3	180 – 200, then 25 min at 200	0.70 ± 0.13 [b]	38
Control		2.17 ± 0.26	
F3a	180 – 200	1.70 ± 0.26 [b]	22
F3b	5 min at 200	1.54 ± 0.23 [b]	29
F3c	20 min at 200	2.4 ± 0.41	0

[a] Temperature range in which fractions were eluted during gas chromatographic separation – *Temperaturbereich, in dem die Fraktionen während der Gaschromatographie eluiert wurden – Ecarts de température pendant l'élution des fractions par chromatographie en phase gazeuse*

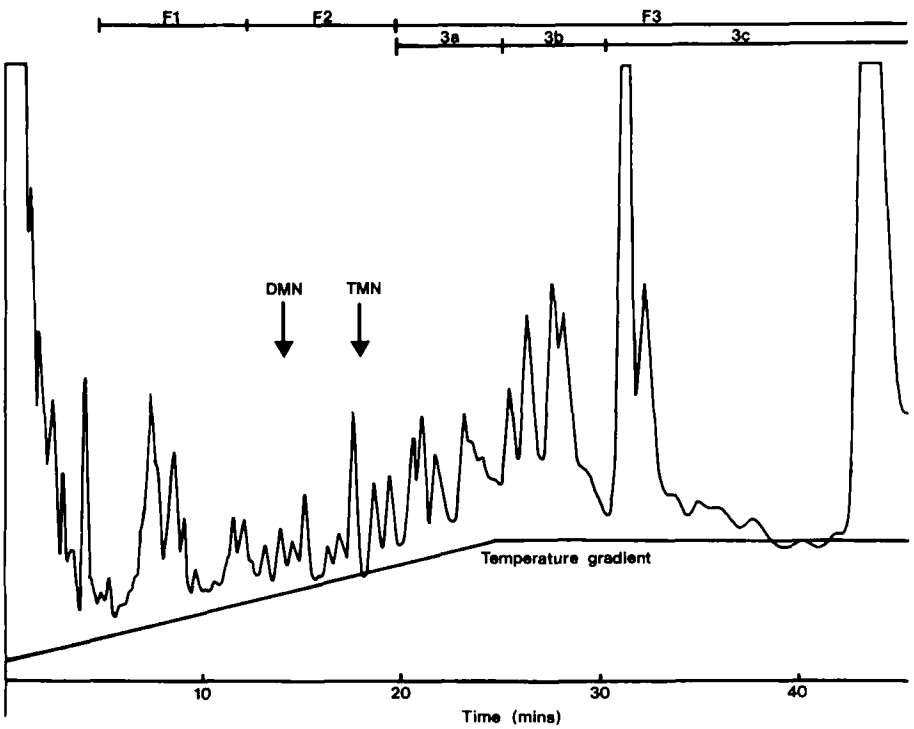
[b] Significantly different from the control ($P < 0.01$) – *Signifikant unterschiedlich von der Kontrolle ($P < 0,01$) – Significativement différent du témoin ($P < 0,01$)*

[1] *Fraktion Nr. – Numéro de la fraction;* [2] *Temperaturbereich – Ecarts de température;* [3] *Wachstumsrate/Tag – Taux de croissance/jour;* [4,5] *Siehe Tabelle 1 – Voir tableau 1*

Tabelle 2. Wachstumshemmende Wirkung der flüchtigen Substanzen im Triebspitzen-Biotest nach gaschromatischer Fraktionierung über Carbowax 20M.

Tableau 2. Activité inhibitrice de la croissance des sous-fractions volatiles dans un essai biologique sur extrémités de pousses après séparation par chromatographie sur Carbowax 20M.

Fig. 1. Separation of volatile components evolved by stored potato tubers by gas chromatography on Carbowax 20M. Temperature gradient 100–200 °C at 4 °C/min, then isothermal at 200 °C for 20 min. Fractions F1–F3, and subfractions F3a–F3c were collected as described in section Materials and methods.



DMN = 1,4-dimethylnaphthalene
TMN = 1,4,6-trimethylnaphthalene

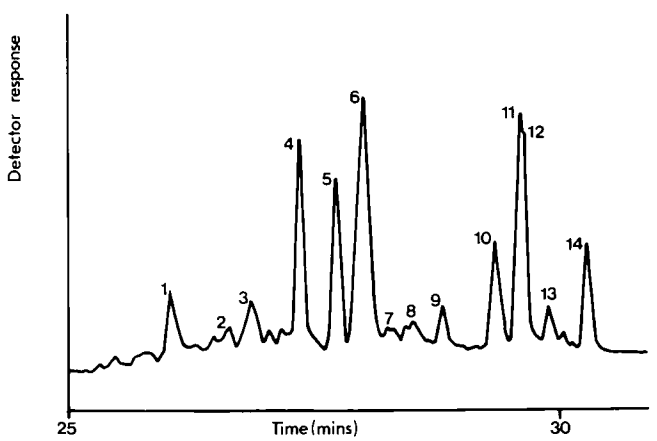
Time (min) – *Zeit (min)* – *Temps (min);* Temperature gradient – *Temperaturprogramm* – *Gradient de températures*

Abb. 1. Abtrennung flüchtiger Komponenten, die von gelagerten Kartoffeln erzeugt wurden, mit Hilfe der Gaschromatographie über Carbowax 20M. Temperaturprogramm 100–200 °C mit 4 °C/min; 20 min isotherm bei 200 °C. Die Sammlung der Fraktionen F1–F3 und die der Unterfraktionen F3a–F3c wurde im Abschnitt Material und Methoden beschrieben.
Fig. 1. Séparation des composés volatiles dégagés par les tubercules de pomme de terre en conservation, par chromatographie en phase gazeuse sur Carbowax 20M. Gradient de températures 100–200 °C à 4 °C/min, isothermique à 200 °C pendant 20 min. Les fractions F1–F3 et sous-fractions F3a–F3c sont récupérées comme décrit au chapitre Matériel et méthodes.

m/e 198, 77, 141, 184, 241. Peaks 11 and 12 are sulphur-containing compounds, and a possible interpretation of the mass spectrum would indicate naphthothiophenes. However, although authentic 1,2-, 2,1-, and 2,3-naphthothiophenes had GC retention times in the same region as peaks 11 and 12, their mass spectra were different. It was not possible to identify the components of peaks 6, 11 and 12 by MS alone and we were unable to apply other analytical techniques such as nuclear magnetic resonance (NMR) or infra-red spectroscopy (IR), owing to the small amounts of the compounds available.

Some of the peaks shown in Fig. 2 were present in the control sample and represent contamination of the volatile fraction introduced during the various manipulations. For instance, peak 4 is a phthalate derivative from plastic materials.

Fig. 2. Computer-reconstructed gas-chromatographic separation of combined subfractions F3a and F3b (Fig. 1) using a capillary column of Carbowax 20M. Gas-chromatographic conditions as stated in section Materials and methods. (Peak numbers see 'Results'.)

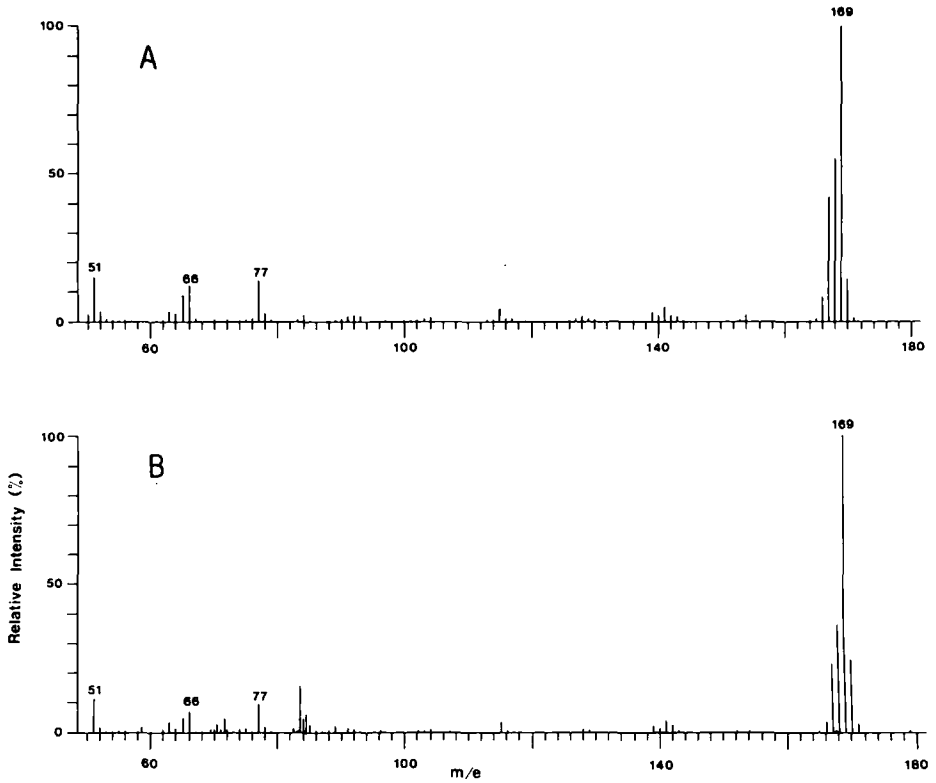


Detector response – *Detecıorsignal – Signal du détecteur;* Time (min) – *Zeit (min) – Temps (min)*

Abb. 2. Computer-aufbereitete gaschromatographische Auftrennung der kombinierten Unterfraktionen F3a und F3b (Abb. 1) mit Hilfe einer mit Carbowax 20M gefüllten Kapillarsäule. Die Bedingungen für die Gaschromatographie sind im Abschnitt 'Material und Methoden' angegeben. (Gipfel-Nr. siehe 'Ergebnisse'.)

Fig. 2. Reconstitution par ordinateur des sous-fractions F3a et F3b (fig. 1) mélangées, après séparation sur colonne capillaire de Carbowax 20M. Les conditions de la chromatographie en phase gazeuse sont mentionnées au chapitre Matériel et méthodes. (Nombre de pics: voir Résultats.)

Fig. 3. Comparison of the mass spectrum of authentic diphenylamine (A) with that given by peak 5 (B) obtained during chromatography on a capillary column of Carbowax 20M (see Fig. 2).



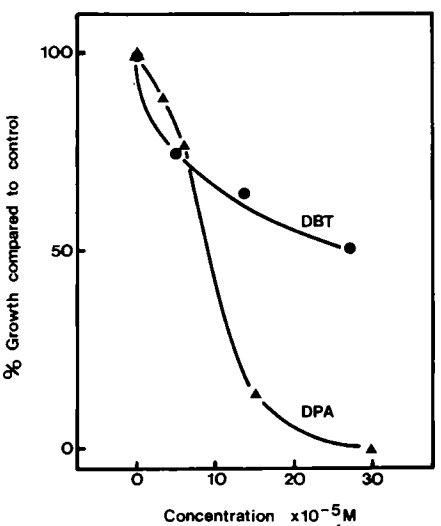
Relative intensity – *Relative Intensität* – *Intensité relative*

Abb. 3. Vergleich des Massenspektrums vom authentischen Diphenylamin (A) mit demjenigen des Gipfels 5 (B), der bei der Chromatographie über eine Kapillarsäule mit Carbowax 20M erhalten wurde (vergl. Abb. 2).
Fig. 3. Comparaison entre le spectre de la diphénylamine pure (A) et celui obtenu à partir du composé issu du pic 5 (B), après chromatographie sur colonne capillaire de Carbowax 20M (voir fig. 2).

The two compounds positively identified in the active subfraction, were tested for sprout-growth-inhibitory activity in the shoot-tip bioassay (Fig. 4). DPA showed significant inhibitory activity while DBT was much less active. Fig. 5 shows a comparison of the sprout growth activity of DPA with 3-methyldiphenylamine (MDPA) and 1,4-dimethylnaphthalene (DMN). The figures show that all three compounds have similar inhibitory activity with concentrations to give a 50 % inhibition of growth of 10, 6 and 8×10^{-5} mol/l respectively for DPA, MDPA and DMN.

In view of the relatively high activity of DPA in the bioassay it was further tested for sprout-growth-inhibitory activity with whole tubers. Table 3 shows results from whole-tuber assays with DPA applied in acetone by spraying or by painting; both methods gave good control of sprout growth. A concentration of between

Fig. 4. Growth inhibition by diphenylamine (DPA) and dibenzothiophene (DBT) in the shoot-tip bioassay.



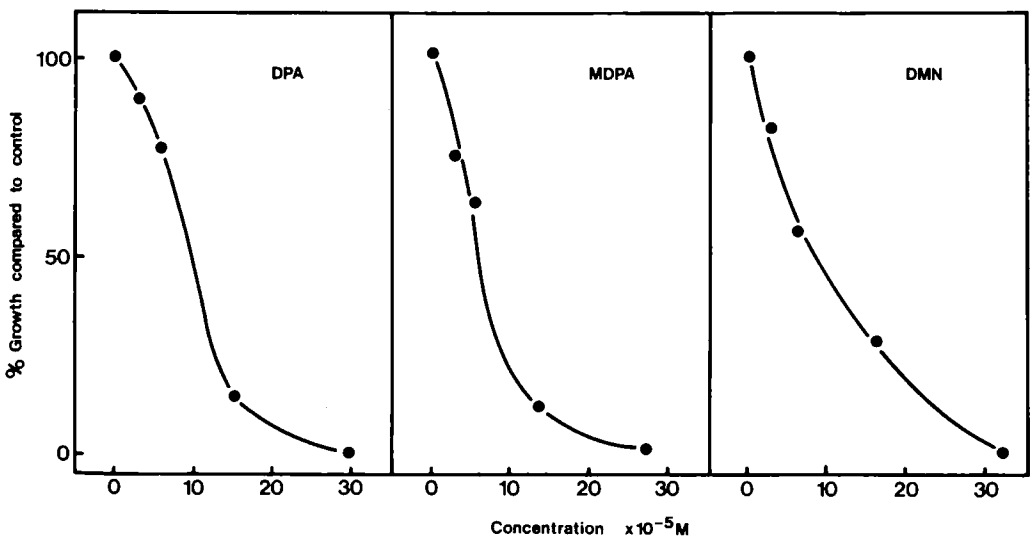
% growth compared to control – *% Wachstum im Vergleich zu Kontrolle – % de croissance par rapport au témoin;* Concentration – *Konzentration – Concentration*

Abb. 4. Wachstumshemmung durch Diphenylamin (DPA) und Dibenzothiophen (DBT) im Triebspitzen-Biotest.
Fig. 4. Inhibition de croissance donnée par la diphénylamine (DPA) et le dibenzothiophène (DBT) dans un essai biologique sur extrémités de pousses.

2–5 mg/kg was sufficient to give 50 % inhibition of sprout growth and near complete control was obtained at a concentration of more than 20 mg/kg.

Trials with up to 500 kg of tubers in specially designed storage cabinets (Filmer & Rhodes, 1983) were used to investigate the problem of applying DPA to whole tubers. Tables 4 and 5 show the results of two different methods of application of DPA. In the first experiment (Table 4), DPA, applied at 72 mg/kg as a fog into the storage cabinet, controlled sprout growth for 38 days, and although the inhibitory effect was still evident after 146 days the degree of control was unsatisfactory. The residue level of DPA at the end of the storage period varied from 4–8 mg/kg but much variation from tuber to tuber, and in the degree of sprout growth also, indicating a very uneven distribution of DPA. Inspection of the false bottom of the cabinet

Fig. 5. Comparison of the growth-inhibitory activities of DPA (diphenylamine), 3-methyldiphenylamine (MDPA) and 1,4-dimethylnaphthalene (DMN) in the shoot-tip bioassay.



Growth compared to control, Concentration – *Siehe Abb. 4 – Voir Fig. 4*

Abb. 5. Vergleich der wachstumshemmenden Wirkung von DPA (Diphenylamin), 3-Methyldiphenylamin (MDPA) und 1,4-Dimethylnaphtalen im Triebspitzen-Biotest.
Fig. 5. Activités inhibitrices de la croissance comparées de la diphénylamine (DPA), du 3-méthyldiphénylamine (MDPA) et du 1,4-diméthylnaphtalène (DMN) dans un essai biologique sur extrémités de pousses.

into which the fog was injected suggested that much of the compound did not reach the tubers.

The second experiment, using DPA-alumina dust, was carried out late in the season (March) with tubers which had been held at low temperature to retard sprouting and which, on transfer to 10 °C, had a high potential for rapid sprout growth and so provided a severe test for a sprout suppressant. The results (Table 5) show that DPA at 100 mg/kg significantly inhibited sprouting for 14 weeks. The distribution of the compound was relatively even between the various layers of tubers with residue values between 7–11 ppm.

Table 3. Effect of DPA (Diphenylamine) on sprout growth in a whole-tuber bioassay. Solutions of DPA in acetone applied to the surface of unsprouted tubers either by spraying (A) or by painting (B). Tubers stored at 10 °C for 7 weeks and the increase in sprout weight determined. Control treatments in which tubers were treated with acetone were carried out.

DPA treatment (mg/kg tuber)[1]	Sprout wt[a,2] (g ± s.e.m.)	Inhibition[3] (%)
A		
Control[4]	4.6 ±0.43	0
5	1.6 ±0.2	65
11	0.6 ±0.13	87
25	0.2 ±0.09	95
40	0.1 ±0.09	98
B		
Control	1.9 ±0.28	0
2	1.1 ±0.24	42
5	0.6 ±0.13	70
10	0.4 ±0.09	81
20	0.04±0.02	98

[a] Mean sprout weight per tuber – *Mittleres Keimgewicht pro Knolle – Poids moyen de germes par tubercule*

[1] *DPA-Behandlung (mg/kg Knollen) – Traitement au DPA mg/kg de tubercule;* [2] *Keimgewicht – Poids de germe;* [3,4] *Siehe Tabelle 1 – Voir tableau 1*

Tabelle 3. Die Wirkung des DPA (Diphenylamin) auf das Keimwachstum in einem Biotest mit ganzen Knollen. In Aceton gelöstes DPA wurde entweder durch Besprühen (A) oder durch Bestreichen (B) auf die Oberfläche ungekeimter Knollen gebracht. Die Knollen wurden bei 10 °C 7 Wochen gelagert und die Zunahme des Keimgewichtes bestimmt. In den Kontrollen wurden die Knollen nur mit Aceton behandelt.

Tableau 3. Effet de la diphénylamine (DPA) sur la croissance du germe dans un essai biologique sur tubercule entier. Solutions de DPA dans l'acétone appliquées à la surface de tubercules non germés par pulvérisation (A) ou au pinceau (B). Tubercules conservés à 10 °C pendant 7 semaines dont l'évolution du poids des germes a été déterminée. Les traitements témoins dans lesquels les tubercules ont été traités à l'acétone ont été éliminés.

Table 4. Effect of DPA (diphenylamine) applied as a 'fog' in acetone on sprout growth in potato tubers stored for 21 weeks at 10 °C. Treatment: 72 mg DPA/kg tubers.

Days in store[1]	Sample site[2]	Control[3]				DPA treatment[8]			
		Av. tuber weight[4] (g)	Av. sprout length[a,5] (cm ± s.e.m.)	Av. sprout weight[b,6] (g ± s.e.m.)	Residue values[c,7]	Av. tuber weight (g)	Av. sprout length[a] (cm ± s.e.m.)	Av. sprout weight[b] (g ± s.e.m.)	Residue values[c]
38	top[9]	155	5.3 ± 0.2	3.5 ± 0.4	0	140	1.0 ± 0.2	0.2 ± 0.05	46
146	top	163	21 ± 0.8	20.4 ± 1.1	0	154	4 ± 0.2	4.2 ± 0.3	8
	middle[10]	156	18 ± 1.1	13.6 ± 0.9	0	156	6 ± 0.3	5.3 ± 0.4	4
	bottom[11]	156	14.5 ± 1.1	13.4 ± 0.9	0	165	7 ± 0.5	6.5 ± 0.6	6
	overall[12]	158	19 ± 0.6	16.9 ± 1.0	0	158	6 ± 0.2	5.3 ± 0.3	6

Results from treated samples are significantly different from the control ($P < 0.01$) – *Die Ergebnisse von den behandelten Knollen unterschieden sich signifikant von denen der Kontrolle ($P < 0,01$) – Les résultats obtenus avec les échantilons traités sont significativement différents du témoin ($P < 0,01$).*

[a] Average length of longest sprout of each tuber – *Mittlere Länge des von jeder Knolle jeweils längsten Keimes – Longueur moyenne du plus long germe de chaque tubercule.*

[b] Average total sprout weight/tuber – *Mittleres Gesamtgewicht pro Knolle – Poids total moyen de germes/tubercule.*

[c] Residue values are expressed as mg/kg fresh weight of tubers – *Rückstandswerte in mg/kg Knollenfrischgewicht – Les valeurs résiduelles sont exprimées en mg/kg de poids frais de tubercules.*

[1] *Tage im Lager – Jours de conservation;* [2] *Lage der Probe – Localisation sur l'échantillon;* [3] *Kontrolle – Témoin;* [4] *Mittleres Knollengewicht – Poids moyen du tubercule;* [5] *Mittlere Keimlänge – Longueur moyenne du germe;* [6] *Mittleres Keimgewicht – Poids moyen du germe;* [7] *Rückstandswerte – Valeur résiduelle;* [8] *DPA-Behandlung – Traitement au DPA;* [9] *Oberer Bereich – Sommet;* [10] *Mittlerer Bereich – Milieu;* [11] *Unterer Bereich – Base;* [12] *Insgesamt – Ensemble*

Tabelle 4. Die Wirkung des in Aceton gelösten und als 'Nebel' applizierten DPA (Diphenylamin) auf das Keimwachstum an Kartoffelknollen, die 21 Wochen bei 10 °C lagerten. Behandlung: 72 mg DPA/kg Knollen.

Tableau 4. Effet de la diphénylamine (DPA) dans l'acétone, appliquée par brumisation, sur la croissance des germes de pommes de terre conservés pendant 21 semaines à 10 °C. Traitement: 72 mg de DPA/kg de tubercules.

Table 5. Effect of DPA (diphenylamine) applied as a surface dusting, on sprout growth in potato tubers stored for 14 weeks at 10 °C. Treatment: 100 mg DPA/kg tubers.

Days in store[1]	Sample site[2]	Control[3]				DPA treatment[8]			
		Av. tuber weight[4] (g)	Av. sprout length[a,5] (cm ± s.e.m.)	Av. sprout weight[b,6] (g ± s.e.m.)	Residue values[c,7]	Av. tuber weight (g)	Av. sprout length[a] (cm ± s.e.m.)	Av. sprout weight[b] (g ± s.e.m.)	Residue values[c]
34	top[9]	148	6 ±0.4	2.7±0.3	0	156	3 ±0.1	0.9±0.1	14
60	top	163	10.5±1.5	8.9±1.8	0	153	3 ±0.1	2.2±0.2	13
102	top	142	8 ±0.4	6.1±0.4	0	158	3.5±0.1	3.0±0.1	7
	middle[10]	161	16.5±1.5	11.8±0.8	0	159	5 ±0.2	3.5±0.3	10
	bottom[11]	155	22 ±0.8	15.8±0.9	0	170	4 ±0.2	2.6±0.2	11
	overall[12]	153	16.5±0.7	12.0±0.6	0	163	4 ±0.1	3.0±0.1	10

Results from treated samples are significantly different from the control ($P<0.01$) – *Die Ergebnisse von den behandelten Knollen unterscheiden sich signifikant von denen der Kontrolle ($P<0,01$) – Les résultats obtenus avec les échantillons traités sont significativement différents du témoin ($P<0,01$).*

[a,b,c] As Table 4 – *Siehe Tabelle 4 – Comme dans le tableau 4*

[1–12] *Siehe Tabelle 4 – Voir tableau 4*

Tabelle 5. Wirkung des DPA (Diphenylamin), nach Bestäuben der Oberfläche, auf das Keimwachstum an Kartoffelknollen, die 14 Wochen bei 10 °C lagerten. Behandlung: 100 mg DPA/kg Knollen.

Tableau 5. Effet de la diphénylamine (DPA) appliquée par poudrage sur la croissance des germes de tubercules conservés pendant 14 semaines à 10 °C. Traitement: 100 mg de DPA/kg de tubercules.

Discussion

The observation that when potato volatile compounds are allowed to accumulate around tubers, sprouting is inhibited, has stimulated research into the chemical nature and biological properties of components of the volatile fraction. The object of the work was to find novel, natural sprout-growth inhibitors which could ultimately be used commercially. In approaching the problem, it was hoped that the observed inhibition might be due to a single compound (or two or more closely related ones) of high sprout-suppressant activity. Previous work (Meigh et al., 1973) had shown the presence of a number of different components showing moderate levels of inhibitory activity, none of which alone could account for the inhibitory activity of the total volatile fraction.

We used the potato shoot-tip bioassay to examine the initial fractionation of the mixture of volatile substances obtained from tubers, and a narrow region of the gas-chromatographic separation with high sprout-growth-inhibitory activity was identified. This region was subsequent resolved by capillary-column gas chromatography into 14 well-defined peaks. Of five peaks which gave well-resolved mass spectra, two were positively identified and one of these (DPA) gave activity, in the shoot-tip and whole-tuber bioassays, at least as high as 1,4-dimethylnaphthalene.

The list of endogenous growth inhibitors, found in the potato volatile fraction, which show varying degrees of sprout-growth-inhibitory activity, includes the mono-, di-, and trimethylnaphthalenes and benzothiazole described by Meigh et al. (1973), 1,4,6-trimethylnaphthalene (Filmer & Rhodes, 1983) and the two compounds described in the present paper, diphenylamine and dibenzothiophene. Of these compounds, 1,4- and 1,6-dimethylnaphthalene, 1,4,6-trimethylnaphthalene and diphenylamine have activities at least of the same order as that given by commercial sprout suppressants such as isopropyl-(N-3-chlorophenyl)-carbamate (CIPC) and tecnazene (TCNB). From the known concentration dependence of growth inhibition and of the levels of these compounds produced by potato tubers, it is possible to make calculations of the contributions of individual volatile inhibitors to the degree of inhibition exerted by the unfractionated volatile mixture. Such calculations made for the naphthalenes by Meigh et al. (1973) suggested that the overall inhibition could not be accounted for by the mono- and di-methylnaphthalenes. A similar calculation leads us to the same conclusion for diphenylamine. However, the chemical diversity of compounds in the volatile fraction which show growth-inhibitory properties is surprising. It is likely that they affect growth processes by different mechanisms and either additive or possibly synergistic interactions between them on overall growth may be involved. The inhibition of sprouting caused by the accumulation of volatile compounds may result from interactions between several chemically distinct volatiles, each of moderate growth-inhibitory activity, rather than from very low concentrations of a single powerful inhibitor. We cannot distinguish between these two possibilities but, on balance, favour the former.

The finding that DPA shows activity of the same order as the commercial sprout suppressants CIPC and tecnazene is important because DPA is already used in the food industry to control the storage disorder superficial scald in apples and pears. For example, Johnson et al. (1980) found that 1000–4000 ppm of DPA applied as a dip to Bramley seedling apples completely controlled the disorder during a 241-day period of controlled atmosphere storage. The fact that DPA is already used as a food

additive led us to investigate its sprout-suppression activity in more detail. DPA, which has not been previously reported in potato volatiles, was very effective as an inhibitor when sprayed or painted onto potato tubers but was less so when applied as a fog, probably because it is insufficiently volatile to be applied in this way which proved so successful with naphthalenes (Filmer & Rhodes, 1983). Surface dusting was more successful, and a level of ca 100 mg/kg applied to dormant tubers early in the storage season could be effective. DPA inhibits scald in apples by acting as an antioxidant preventing the oxidation of α-farnesene (Huelin & Coggiola, 1970). Its mode of action in inhibiting sprouting is unknown but its antioxidant properties led us to study other antioxidants used as food additives for sprout-growth-inhibitory properties; none of the compounds studied which included ethoxyquin had significant sprout-growth-inhibitory activity. Our work suggests that DPA is worthy of more detailed consideration as a commercial sprout suppressant and this would necessitate the development of methods for its effective dispersion in potato stores.

Zusammenfassung

Untersuchung keimwachstumshemmender Verbindungen in der flüchtigen Fraktion von Kartoffelknollen

Das Vorkommen natürlicher Keimwachstumshemmer in der flüchtigen Fraktion von Kartoffelknollen ist erneut untersucht worden. Die gesamte flüchtige Fraktion, die eine starke Hemmwirkung in einem Kartoffeltriebspitzen-Biotest (Tab. 1) zeigte, wurde mit Hilfe der Gaschromatographie auf einer mit Carbowax 20M gefüllten Säule abgetrennt. Die chromatographische Abtrennung wurde in 3 Fraktionen aufgeteilt (Abb. 1), die auf ihre wachstumshemmende Wirksamkeit untersucht wurden (Tab. 2). Dabei zeigte eine Fraktion (F3) eine Hemmwirkung des Keimwachstums. Diese Fraktion (Abb. 1) wurde erneut chromatographiert und in drei Unterfraktionen geteilt, die einzeln auf Keimwachstumshemmung untersucht wurden. Die Hemmwirkung wurde den Unterfraktionen F3a und F3b (Tab. 2) zugeordnet. Sie wurden vereinigt und erneut auf einer Kapillarsäule mit Carbowax 20M chromatographiert, wobei mehrere Gipfel auftraten (Abb. 2). Fünf davon ergaben gut definierte Massenspektren. Zwei dieser Verbindungen wurden als Diphenylamin (DPA) und Dibenzothiophen identifiziert (Abb. 3). Die chemische Natur dieser Verbindungen wurde an Hand authentischer Proben dieser Substanzen bestätigt, die identische Retentionszeiten und Massenspektren ergaben.

DPA und Dibenzothiophen wurden dem Biotest unterworfen (Abb. 4). Dabei erwiesen sich beide Verbindungen als wirksam, DPA zeigte dabei jedoch die grössere Aktivität. Für eine 50%ige Wachstumshemmung im Biotest waren etwa 10×10^{-5} mol/l DPA erforderlich, aber 8×10^{-5} mol/l Dimethylnaphtalen und 6×10^{-5} mol/l 3-Methyldiphenylamin (Abb. 5).

Weil DPA ein verhältnismässig wirkungsvoller Hemmstoff für das Keimwachstum ist und auch bereits in einigen Anwendungen als Lebensmittelzusatz zugelassen ist, wurde es auf seine Fähigkeit, die Keimung zu unterdrücken, untersucht. Wenn DPA direkt auf die Knollenoberfläche gebracht wird, hemmt es die Keimung sehr wirkungsvoll (Tab. 3). Für eine 50%ige Hemmwirkung sind weniger als 5 ppm erforderlich, wenn die Substanz entweder in Aceton gelöst und versprüht wird oder wenn sie auf die Knollenoberfläche direkt aufgetragen wird. Konzentrationen von 20 ppm schränken die Keimung nahezu vollständig ein. Wegen der verhältnismässig geringen Flüchtigkeit der Verbindung gibt es jedoch Probleme, sie auf den Kartoffelknollen im Lager zu verteilen. Es wurden 2 Applikationsmethoden geprüft (Tab. 4 und 5). Wurde DPA in Acetonlösung

als Nebel ausgebracht, dann war die Verteilung der Substanz unzulänglich und die Keimunterdrückung ungleichmässig. Bei der zweiten Methode wurde die Substanz mit Tonerde gemischt und über die Knollenoberfläche gestäubt. Sie war vielversprechend, eine Weiterentwicklung dieser Methode ist jedoch erforderlich. Insgesamt weisen die Ergebnisse darauf hin, dass DPA wirkungsvoll die Keimung unterdrückt, und dass die Notwendigkeit besteht, verbesserte Applikationsmethoden für die Anwendung im Kartoffellager zu entwickeln.

Die Beobachtung, dass die Ansammlung flüchtiger Substanzen um die Kartoffelknolle zur Keimhemmung führt, wird im Hinblick auf die vorliegende Arbeit und auf die Ergebnisse früherer Autoren diskutiert. Es wird auch die Möglichkeit erwogen, dass die Wirkung Interaktionen zwischen zwei oder mehreren wachstumshemmenden flüchtigen Substanzen einschliesst.

Résumé

Recherches sur les substances inhibitrices de la germination contenues dans la fraction volatile d'extraits de tubercule de pomme de terre

La présence d'inhibiteurs de la germination dans la fraction volatile des tubercules de pomme de terre a été de nouveau recherchée. La fraction volatile totale, ayant une haute activité inhibitrice dans un essai biologique sur extrémité de pousses (tabl. 1), a été isolée par chromatographie gaz-liquide sur colonne de Carbowax 20M. Trois fractions ont été séparées à partir de cette dernière (fig. 1) et leur activité inhibitrice a été étudiée (tabl. 2); une fraction (F3) a montré une action inhibitrice sur la croissance des germes. Cette fraction (fig. 1) a donné trois sous-fractions par chromatographie dont F3a et F3b (tabl. 2) qui possédaient l'activité recherchée. Ces deux fractions ont été mélangées et séparées de nouveau par chromatographie sur colonne capillaire de Carbowax 20M (fig. 2). Il en est résulté un certain nombre de pics dont 5 avaient un spectre bien défini. Deux substances ont été identifiées comme étant la diphénylamine (DPA) (fig. 3) et le dibenzothiophène. L'anologie chimique de ces composés a été confirmée à l'aide des produits purs qui donnaient le même temps de rétention et le même spectre que les substances alors inconnues.

DPA et dibenzothiophène ont été utilisés dans un essai biologique (fig. 4) et possédaient quelque activité, celle-ci étant beaucoup plus importante pour le DPA. Environ 10×10^{-5} mol/l de DPA sont nécessaires pour inhiber la croissance à 50 % dans l'essai biologique alors que 8×10^{-5} mol/l et 6×10^{-5} mol/l respectivement de diméthylnaphthalène et de 3 méthyldiphénylamine (fig. 5) ont le même effet.

Comme le DPA est un inhibiteur efficace, déjà accepté en tant qu'additif alimentaire dans quelques applications, il a été testé comme inhibiteur de germination. Appliqué directement à la surface des tubercules, le DPA est un inhibiteur de germination très efficace (tabl. 3). Moins de 5 ppm sont nécessaires pour inhiber 50 % de la germination s'il se trouve en solution dans l'acétone et est appliqué par pulvérisation ou au pinceau; à la concentration 20 ppm, il provoque une inhibition presque totale de la germination. Cependant, ce produit étant peu volatile, il pose un problème de dispersion à l'intérieur des tubercules en conservation. Deux méthodes d'application ont été essayées (tabl. 4 et 5); l'application du DPA par brumisation n'a pas donné de bons résultats car sa répartition n'a pas été régulière et le taux d'inhibition n'était pas homogène; mélangé à de l'alumine et appliqué par poudrage à la surface des tubercules, il a donné des résultats encourageants mais qui nécessitent encore des études. En conclusion, le DPA s'avère être un inhibiteur de germination efficace qui demande toutefois encore des recherches sur la méthode adéquate pour obtenir sa dispersion dans les locaux de conservation.

Les effets de l'accumulation de substances volatiles autour des tubercules sur l'inhibition de la germination sont discutés en relation avec le présent travail et les découvertes des premiers chercheurs. La possibilité que ces effets mettent en jeu deux inhibiteurs de croissance ou plus est prise en considération.

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