INDUCED ALKALOID LEVELS IN POTATO TUBERS¹

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Introduction

Adverse biological effects have been attributed to the feeding of plant materials which had been treated with maleic hydrazide (MH) during the growing cycle (4, 14). Two recent toxicological studies with insects (19, 20) failed to confirm such effects following the use of MH. Since potato tubers comprised one of the diets in question, it seemed possible that MH treatments had either induced the formation of some toxic material or had increased the levels of some naturally occurring deleterious substance.

Because the major alkaloids in potatoes, solanine and solanidine, have been described as mitotic poisons (3) as well as cholinesterase inhibitors (11), it seemed worthwhile to investigate the effect of treatment of potatoes with MH on their alkaloid content.

Nicotinic acid (NA) is a known inhibitor of cholesterol synthesis in animals and yeasts (1, 2, 13) and gibberellic acid (GA) has been reported to decrease NA (12). Because of the chemical relationship between sterols and potato alkaloids, the effects of these two substances on alkaloids in tubers were also investigated.

MATERIALS AND METHODS

Field-grown potatoes (Solanum tuberosum L. cv. Kennebec), were sprayed to run-off four weeks before harvest with an aqueous solution of MH-30, the diethanolamine salt, containing 0.75% of the active ingredient. The harvested tubers were stored in the dark at 5C and 80% relative humidity. Tubers used in this investigation were removed after 6 months in storage. Three to five uniform, healthy tubers, each weighing from 130 to 140 grams were washed, dried and weighed for each chemical treatment.

The tubers were immersed in various concentrations of aqueous MH (sodium salt), aqueous NA, aqueous GA and water (see Table I). Penetration of these solutions into the tubers was facilitated by using a reduced-pressure infiltration technique (5) (5 mm Hg for 5 minutes). The infiltrated tubers were placed on trays and kept for 4 days at 10 C and low light intensity (approximately 100 f.c.). Alkaloid contents were determined by both the colorimetric procedure of Hilton and Gamborg (6) and the thin-layer chromatography technique of Schreiber et al. (16). It was established that alkaloid recoveries using these procedures were not affected by the presence of either GA or NA. The field-sprayed potatoes (MHP) were analyzed by the method of Hoffman (7) and were found to contain 58 p.p.m. of MH.

RESULTS AND DISCUSSION

The pre-harvest use of MH-30 on potato plants had negligible effect on the alkaloid content of the stored tubers (Table 1). In agreement

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Table 1.—Alkaloid content of potato tubers infiltrated with maleic hydrazide (MH), nicotinic acid (NA) and gibberellic acid (GA).

Treatment (Alkaloids* mg/100 g fresh wt)
P (control, not field-treated) MHP (field-treated with MH) 1. P infiltrated with H ₂ O 2. MHP infiltrated with H ₂ O 3. P infiltrated with MH (2 x 10 ⁻² M) 4. P infiltrated with MH (4 x 10 ⁻² M) 5. P infiltrated with NA (2 x 10 ⁻² M) 6. P infiltrated with NA (4 x 10 ⁻² M) 7. P infiltrated with GA (4.70 x 10 ⁻³ M)	6.3 14.3 22.9 13.2 10.3 14.4 3.9
8. P infiltrated with GA $(2.89 \times 10^{-4}M)$ 9. P infiltrated with GA $(1.44 \times 10^{-3}M)$	

^{*}Results are averages for duplicate samples each analyzed in triplicate.

with previous observations (9, 17, 18) that increased metabolic activity in tubers was reflected in higher alkaloid contents, a two-fold increase was obtained when the controls (P) were removed from storage and infiltrated with water. The three-fold alkaloid increase obtained for Treatment 2 substantiated Isenberg's (8) findings that MH at relatively low concentrations stimulated respiration and metabolic activity. While MH is known as an effective inhibitor of plant growth, its presence exerted an enhancing effect on the synthesis of alkaloids when the tubers were infiltrated with water. Perhaps this is not too surprising since high concentrations of alkaloids are present in the non-growing periderm and cortical parenchyma tissues of tubers (18) and the strongest inhibitory effect of MH would be expected in the eye regions where cell division occurs. This was confirmed when analysis on MHP eye tissues showed an alkaloid content of 41 mg/100 g as opposed to 53 mg/100 g for the skin tissues.

Infiltration with aqueous solutions of MH (Treatments 3, 4) resulted in slightly less alkaloids than infiltration with water (Treatment 1). This finding is of particular interest in view of the recent work of Franklin and Lougheed (5) who reported on the effectiveness of vacuum infiltration of tubers with solutions of MH for the inhibition of sprouting.

The higher concentration of NA (Treatment 6) resulted in a much lower alkaloid content than water alone (Treatment 1) and in fact was the only treatment that caused a reduction in alkaloid content below that found in non-treated potatoes. This effect may be explained by comparing the structural similarities between potato alkaloids and cholesterol (13) and the fact that NA inhibited both cholesterol synthesis in animal tissues (2, 15) and sterol biosynthesis in Saccharomyces cerevisiae (1). The present work indicates that mechanisms similar to those which inhibit sterol biosynthesis by NA in animals and yeast may be operative in higher plants causing a decrease in those alkaloids which resemble steroids in structure.

Results for treatments 7, 8, 9 showed that increasing GA concentrations caused alkaloid contents of the infiltrated tubers to go through

a maximum. Parallel findings were reported by Smith and Rappaport (17) in that the endogenous GA content of tubers increased on sprouting. Parups (12) also found that GA decreased the level of NA in Nicotiana which bears a close botanical relationship to Solanum. Since NA inhibits the formation of alkaloids in tubers, the overall anticipated effect of GA would be an increase in alkaloids.

Chromatographic analyses (10, 12) showed that the amounts of endogenous NA were relatively large for the controls (P), intermediate for MHP (field-sprayed with MH) infiltrated with water, and lowest for P infiltrated with GA. This order would be expected from the relative levels of alkaloids reported above.

It may be concluded that the field use of maleic hydrazide on potato plants had a negligible effect on the levels of alkaloids in the stored tubers. However, alkaloid synthesis was stimulated in such tubers when the rate of metabolism was increased by infiltration with water.

Depending on the concentration in the infiltrating solution nicotinic acid depressed alkaloid levels in tubers while gibberellic acid stimulated their formation.

Summary

The effect on alkaloids in tubers following treatment of potato plants with maleic hydrazide has been investigated using an infiltration technique. The alkaloid content remained unaltered in the stored tubers but there was an increase in alkaloid levels when the rate of metabolism increased. Nicotinic acid was found to depress alkaloid levels in tubers while gibberellic acid stimulated their formation.

RESUMEN

El efecto sobre los alcoloides en los tubércuols que resulta del tratamiento de plantas con hidrásido maléico ha sido investigado usando un método de infiltración. El contenido en alcaloides quedó sin cambio en tubérculos almacenados, pero hubo un incremento en contenido de alcaloides cuando la tasa de metabolismo aumentó. Se descubrió que el ácido nicotínimo redujo los niveles de alcoloides mientras el ácido giberílico estimuló su producción.

LITERATURE CITED

- Bowen, D. M. and R. E. Olson. 1964. Effect of nicotinic acid on sterol bio-synthesis in Saccharomyces cerevisiae. Nature 203: 762-763.
- 2. Dorfman, R. I. 1963. Inhibitors of steroid actions and cholesterol and steroid biosynthesis, chapt. 15 In Metabolic Inhibitors, [ed.], R. M. Hochster and J. H. Quastel. Academic Press, New York.
- 3. Druckrey, H., P. Dannenberg and D. Schmaehl. 1953. Mitotic poisons. Arzneimittel Forsch. 3: 151-161.
- Fischnich, O., C. Paetzold and C. Schiller. 1958. Wachstumsregulatoren im Kartoffelbau. Eur. Potato J. 1: 25-30.
 Franklin, E. W. and E. C. Lougheed. 1966. A new method for applying maleic
- hydrazide to inhibit sprouting of potato tubers. Can. J. Plant Sci. 46: 450-452.
 6. Hilton, R. J. and O. L. Gamborg. 1957. Factors in relation to tuber quality in potatoes. Can. J. Plant Sci. 37: 407-412.
- Hoffman, I. and R. B. Carson. 1962. Determination and distribution of maleic hydrazide in vegetables and fruits. J. Assoc. Offic. Agr. Chemists 45: 788-789.
 Isenberg, F. M. R. 1954. The effect of maleic hydrazide on plants. Ph.D. Thesis. Penn. State Univ.

- Lampitt, L. H., J. H. Bushill, H. S. Rooke and C. M. Jackson. 1943. Solanine, glycoside of the potato. 2. Its distribution in the potato plant. J. Soc. Chem, Ind. 62: 48-51.
- Leiserson, L. and T. B. Walker. 1955. Paper chromatography of nicotine and related compounds. Anal. Chem. 27: 1129-1130.
 Menn, J. J., J. B. McBain and M. J. Dennis. 1964. Detection of naturally
- occurring cholinesterase inhibitors in several crops by paper chromatography. Nature 202: 697-698.
- Parups, E. V. 1960. Effect of gibberellic acid applications to leaves of Nicotiana on nornicotine, anabasine, metanicotine, oxynicotine and nicotinic acid content. Tobacco Sci. 4: 163-165.
- Richards, J. H. and J. B. Hendrickson. 1964. The biosynthesis of steroids, terpenes and acetogenins. W. A. Benjamin, Inc., New York.
- Robinson, A. G. 1960. Effect of maleic hydrazide and other plant growth regulators on the pea aphid, Acyrthosiphon pisum (Harris), caged on broad bean, Vicia faba L. Can. Entomologist 92: 494-499.
- Schoen, H. 1958. Effect of nicotinic acid on the cholesterol contents of rat 15. liver. Nature 182: 534.
- Schreiber, K., O. Aurich and G. Osske. 1963. Solanum-Alkaloide 18. Duenn-schichtchromatographie von Solanum-Steroidalkaloiden und Steroid-sapogeninen. J. Chromatog. 12: 63-69. Smith, O. E. and L. Rappaport.
- 1961. Endogenous gibberellins in resting and sprouting potato tubers. Adv. Chem. 228: 42-48.
 Wolf, M. J. and B. M. Duggar. 1946. Estimation and physiological role of
- solanine in the potato, J. Agr. Research 73: 1-32.
- Yule, W. N., E. V. Parups and I. Hoffman. 1966. Toxicology of plant-translocated maleic hydrazide. Lack of effects on insect reproduction. J. Agr. Food
- Chem. 14: 407-409. Yule, W. N., I. Hoffman and E. V. Parups. 1966. Insect toxicological studies 20. of maleic hydrazide translocated in the potato plant. Bull. Environ. Contam. Toxicol. 1: 251-256.