Effect of Foliar Application of Indoleacetic Acid on the Total Glycoalkaloids and Nitrate Nitrogen Content of Potatoes

Rathy Ponnampalam and Nell I. Mondy*

Katahdin and Kennebec potatoes grown at the Cornell Vegetable Research Farm at Freeville, NY, during two growing seasons were used in this study. The effect of foliar application of indoleacetic acid (IAA) on the total glycoalkaloid (TGA) and nitrate N (NO_3 N) content of Katahdin and Kennebec potatoes was examined. The Kennebec variety was higher in TGA content than Katahdin, and in both years the TGA content of both varieties was significantly decreased ($p < 0.05$) following the use of IAA. Year-to-year variation in TGA content was observed in both varieties. Katahdin was higher in NO_3 N content than the Kennebec variety, and tubers from plants treated with IAA were significantly ($p < 0.05$) lower in NO_3 N content than the control. Cortex tissue was significantly ($p < 0.05$) higher in NO_3 N content than pith tissue. Significant year-to-year variation in NO_3 N content was observed.

INTRODUCTION

Auxins such as indoleacetic acid (IAA) are naturally occurring hormones that promote plant growth. The effect of auxins is to promote cell enlargement or cell elongation.

The presence of IAA and other auxins in potato tubers has been reported by several workers (Booth and Wareing, 1958; Montuella and Cornette, 1964). Synthetic auxins have been shown to (1) increase yield (Gruodiene, 1963; Kiryukhin, 1969), (2) improve quality (Prince and Blood, 1949) and intensify red skin color (McCubbin, 1957), (3) control weeds (Prince and Blood, 1949), and (4) inhibit sprouting (Smith et al., 1949). Foliar application of IAA to Katahdin potato plants increased the phenolic content, enzymatic discoloration, total and nonprotein nitrogen, free amino acids, crude lipid and phospholipid content of tubers and decreased the iron content of the tubers (Chandra and Mondy, 1981).

The glycoalkaloid and nitrate N (NO_3 N) contents of potato tubers are also important attributes when assessing tuber quality. Glycoakloids, a class of naturally occurring toxicants, have been associated with bitter flavor (Sinden et al., 1976), inhibition of cholinesterase, and poisoning in humans and farm animals (McMillan and Thompson, 1979; Bomer and Mattis, 1924).

The TGA content of potatoes depends on variety, maturity, environmental factors, and stress conditions (Sinden and Webb, 1974; Locci and Kuc, 1967) and the use of sprout inhibitors (Mondy et al., 1978: Wu and Salunkhe, 1977). It is recommended that potatoes should contain no more than 20 mg/100 g of fresh weight (Bomer and Mattis, 1924).

High concentrations of nitrates in our food supply are of great concern because of their precursor role in the formation of nitrites. Nitrates have been shown to react with secondary and tertiary amines to form carcinogenic and mutagenic *N*-nitroso compounds (Walter et al., 1979), and investigations have been undertaken to examine the possible role of nitrate and nitrite in the incidence of human cancer (Swann, 1975) and infant methaemoglobinamia (Phillips, 1971). The nitrate and nitrite contents of human saliva can be correlated with nitrate and nitrite intake (Demkowicz-Dobrzanski et al., 1982). The nitrate content of vegetables varies, and according to White (1975), potatoes contribute approximately 14% of the per capita injestion of nitrates in the United States. The nitrate content of potatoes is influenced by variety, type, and amount of nitrogen fertilizer, climate, moisture stress, and storage conditions (Augustin et al., 1977; Carter and Bosma, 1974). Carter and Bosma (1974) found greater concentrations of NO_3 N located below the periderm of the tuber. No studies were found concerning the effect of the auxin on nitrates in potatoes, but Stahler and Whitehead (1950) found that sugar beet leaves sprayed with the synthetic auxin 2,4-D contained 20 times more nitrate than the controls.

The present investigation was undertaken to study the effect of foliar application of IAA on the TGA and NO_3 N contents of potato tubers.

MATERIALS AND METHODS

Katahdin and Kennebec potatoes grown at the Cornell Vegetable Research Farm at Freeville, N.Y. during two growing seasons were used in this study. At the beginning of flowering (approximately 60 days after planting) and again after a 1-week interval the plants were sprayed with IAA (10^{-5} M) dispersed in methanol (Sigma Chemical). Controls were sprayed with methanol alone. The randomized block design contained two replicated plots per treatment. Potatoes were harvested 20 weeks after planting and stored at 5 °C for 10 days before analysis.

Tubers of medium size were selected from both the auxin-treated and the control groups. Tubers were cut longitudinally from bud to stem end in order to include both apical and basal portions, and slices were subsequently separated into cortex (including the periderm) and pith sections, frozen, lyophilized in a Stokes freeze dryer, ground in a Wiley mill through a 40-mesh screen, and stored under nitrogen until analyzed. The cortex tissue (including periderm) was used for glycoalkaloid determination since this is the area highest in metabolic activity and the area known to be highest in glycoakaloid content (Wolf and Duggar, 1940). In each year of the study both varieties were sampled twice. Each sample represented 10 tubers, and duplicate determinations were made on each sample.

Total Glycoalkaloid Determination. Total glycoalkaloid content (TGA) was determined by using the modified titration method of Bushway et al. (1980). Potato powders were hydrated for 1 h (1:4, powder/water, w/v) before extraction of glycoalkaloids (Mondy and Ponnamplam, 1983) with chloroform/methanol bisolvent mixture (1:2, v/v). This was followed by precipitation of TGA in concentrated ammonium hydroxide and quantified by

Department of Food Science (R.P.) and Institute of Food Science and Division of Nutritional Sciences (N.I.M.), Cornell University, Ithaca, New York 14853.

0021-8561/86/1434-0686$01.50/0

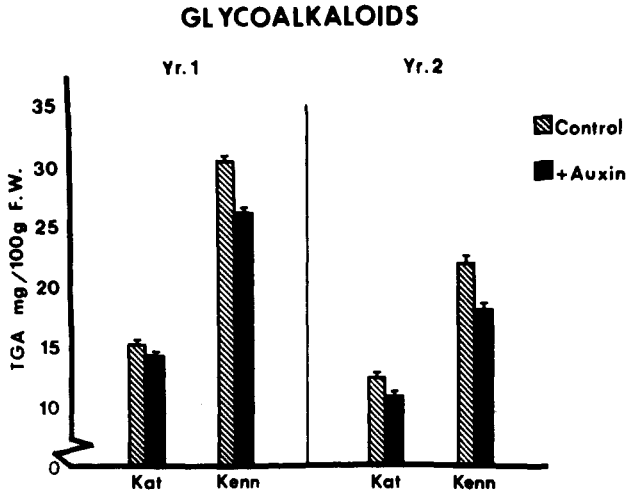

Figure 1. Effect of IAA, an auxin, on total glycoalkaloid content of Katahdin and Kennebec potato tubers grown during two seasons.

nonaqueous titration (Fitzpatrick et al., 1978).

Nitrate Nitrogen. NO_3 N content was determined by the method of Ulrich et al. (1959) using phenoldisulfonic acid on an aqueous extract of freeze-dried powders.

Statistical Analysis. Statistical significance of the data was determined by using 2 × 2 × 2 factorial analysis of variance with protected LSD test described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Total Glycoalkaloid. In both years, Katahdin and Kennebec tubers from plants treated with IAA were significantly ($p < 0.05$) lower in TGA content than the controls (Figure 1). Kennebec was significantly ($p < 0.05$) higher in TGA content than the Katahdin variety. Year-to-year variation in TGA content was observed in both varieties.

Although this study found the TGA content of Kennebec cortex tissue to exceed the recommended safe limit (20 mg/100 g fw), it is unlikely that this level would be toxic, since the cortex tissue comprises only 20–40% by weight of the whole tuber and both cortex and pith tissues will be consumed when tubers are eaten.

In both years of the study, foliar application of IAA inhibited significantly the accumulation of glycoalkaloids in tubers. Wu and Salunkhe (1977) observed the inhibition of wound-induced glycoalkaloid formation in potato tubers by as much as 70–86% using the sprout inhibitor CIPC. Foliar application of sprout inhibitor MH significantly reduced the glycoalkaloid content of Katahdin and Kennebec tubers (Mondy et al., 1978). Leopold and Klein (1952) showed that MH and IAA are competitive inhibitors, and Brian and Hemming (1957) found MH to be a gibberellin antagonist. Both IAA and MH inhibit glycoalkaloid formation in tubers, but the mechanism by which these two reduce glycoalkaloid synthesis may be different. Previous work in our laboratory has shown that IAA and MH differ in their effect on other constitutents of potatoes such as lipids and phenols. IAA increased the crude and phospholipid contents, while MH decreased these contents in the tubers. Both IAA and MH increased the phenolic content of the tubers (Chandra and Mondy, 1981; Mueller and Mondy, 1977).

Nitrate Nitrogen. In both years tubers from plants treated with IAA were significantly ($p < 0.05$) lower in NO_3 N content than the controls (Figure 2). Significant ($p < 0.05$) varietal and year-to-year variation in NO_3 N content was observed. Katahdin was higher in NO_3 N content than

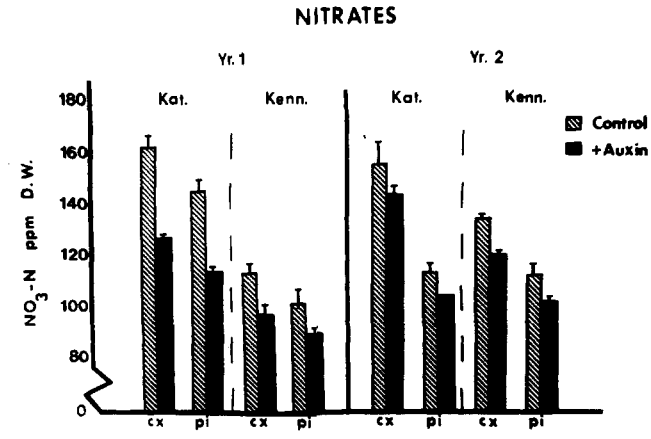

Figure 2. Effect of IAA, an auxin, on nitrate N content of Katahdin and Kennebec potato tubers grown during two seasons: cx, cortex; pi, pith.

Kennebec. The decrease in NO_3 N of tubers from plants treated with IAA was probably due to nitrogen transformation since Chandra and Mondy (1981) observed an increase in total and nonprotein nitrogen and free amino acids in Katahdin tubers from IAA-treated plants. Auxins accelerate protein synthesis in a variety of systems via the mechanism of enhanced transcription and translation (Maas and Klambt, 1977; Simpson and Torrey, 1977).

Cortex tissue was significantly ($p < 0.05$) higher in NO_3 N content than pith tissue. This was true for the control as well as tubers from plants treated with IAA.

CONCLUSION

In both years foliar application of an auxin, IAA, inhibited significantly ($p < 0.05$) glycoalkaloid formation in Katahdin and Kennebec tubers. The Kennebec variety was significantly ($p < 0.05$) higher in TGA content than Katahdin. Year-to-year variation in TGA content was observed in both varieties. Katahdin and Kennebec tubers from plants treated with IAA were significantly ($p < 0.05$) lower in NO_3 N content than the controls. Significant varietal and year-to-year variation in NO_3 N content was observed.

ACKNOWLEDGMENT

We acknowledge the assistance of Professor Joseph B. Sieczka, Department of Vegetable Crops, for his help in growing and harvesting of the potatoes.

Registry No. IAA, 87-51-4; N_2, 7727-37-9; NO_3^-, 14797-55-8.

LITERATURE CITED

Augustin, J.; McDole, R. E.; Painter, G. C. *Am. Potato J.* **1977**, *54*, 125.
Bomer, A.; Mattis, H. *Z. Unters. Nahr.-Genussm. Gebrauchsgegenstaende* 1924, *47*, 97.
Booth, A.; Wareing, P. F. *Nature* **1958**, *182*, 406.
Brian, P. W.; Hemming, H. G. *Ann. Appl. Biol.* **1957**, *45*, 489.
Bushway, R. J.; Wilson, A. M.; Bushway, A. A. *Am. Potato J.* **1980**, *57*, 561.
Carter, J. N.; Bosma, S. M. *Agron. J.* **1974**, *66*, 263.
Chandra, S.; Mondy, N. I. *J. Food Sci.* **1981**, *46*, 1970.
Demkowicz-Dobrzanski, K.; Jasinska, G.; Wojciechowska, E.; Wronkowski, Z. *Bromatol. Chem. Toksykol.* **1982**, *15*, 299.
Fitzpatrick, T. J.; Mackenzie, J. D.; Gregory, P. *Am. Potato J.* **1978**, *55*, 247.
Gruodiene, J. *Lietuvos TSR Aukstuju Mokyklu Mokslo Darbai, Biol.* **1963**, *3*, 31.
Kiryukhin, V. P. *Khim. Sel. Khoz.* **1969**, *7*, 215.
Leopold, A. C.; Klein, W. H. *Physiol. Plant.* **1952**, *5*, 91.
Locci, R.; Kuci, J. *Phytopathology*, **1967**, *57*, 1271.
Maas, H.; Klambt, D. *Planta*, **1977**, *133*, 117.
McCubbin, E. N. *Proc. Fla. State Hortic. Soc.* **1957**, *70*, 93.
McMillan, M.; Thompson, J. C. *Q. J. Med.* **1979**, *48*, 227.

Mondy, N. I.; Ponnampalam, R. *J. Food Sci.* **1983**, *48*, 612.
Mondy, N. I.; Tymiak, A.; Chandra, S. *J. Food Sci.* **1978**, *43*, 1033.
Montuella, B.; Cornette, J. *Eur. Potato J.* **1964**, *7*, 161.
Muller, T. O.; Mondy, N. I. *J. Food Sci.* **1977**, *42*, 618.
Phillips, W. E. J. *Food Cosmet. Toxicol.* **1971**, *9*, 219.
Prince, F. S.; Blood. P. T. *Agron. J.*, **1949**, *41*, 219.
Simpson, S. F.; Torrey, J. G. *Plant Physiol.* **1977**, *59*, 4.
Sinden, S. L.; Deahl, K. L.; Aulenback, B. B. *J. Food Sci.* **1976**, *41*, 520.
Sinden, S. L.; Webb, R. E. *U.S.D.A. Tech. Bull.* **1974**, 1472.
Smith, O.; Ellison, J. H.; McGoldrick, F. *Science (Washington, D.C.)* **1949**, *109*, 66.
Stahler, L. M.; Whitehead, E. I. *Science (Washington, D.C.)* **1950**, *112*, 749.
Steel, R. G. B.; Torrie, J. H. *Principles and Procedures of Statistics*; McGraw-Hill: New York, 1980.
Swann, P. F. *J. Sci. Food Agric.* **1975**, *26*, 1761.
Ulrich, A.; Rieri, D.; Hills, F. J.; George, A. G.; Moore, M. D.; Johnson, C. M. *Bull.—Calif. Agric. Exp. Stn.* **1959**, 766.
Walters, C. L.; Carr, F. P. A.; Dyke, C. S.; Saxby, M. J.; Smith, P. L. R. *Food Cosmet. Toxicol.* **1979**, *17*, 473.
White, J. W. Jr. *J. Agric. Food Chem.* **1975**, *23*, 886.
Wolf, M. J.; Duggar, B. M. *Am. J. Bot.* **1940**, *27*, 20.
Wu, M. T.; Salunkhe, D. K. *J. Food Sci.* **1977**, *42*, 622.

Received for review August 8, 1984. Revised manuscript received September 9, 1985. Accepted March 18, 1986.

Metabolism of the Synthetic Prostaglandin Alfaprostol in the Cow

Maurice Kaykaty,* George Weiss, and Michael Barbalas

The major metabolites formed from studies of alfaprostol in the cow and in vitro have been investigated. Incubation of 15-[^{14}C]alfaprostol (18,19,20-trinor-17-cyclohexyl-13,14-didehydro-PGF$_{2\alpha}$ methyl ester) with 9000*g* bovine liver supernatant yielded mainly one metabolite, alfaprostol acid (I), which is formed by hydrolysis of the methyl ester. This was also the main metabolite found in cow injection site muscle at 24 h after dosing (97–98%) and 5 days after dosing (66%). It was not detected in urine. The major pathway of alfaprostol metabolism in the cow is by way of β-oxidation following deesterification. The major metabolites identified in cow urine were dinor-5,6-dihydroalfaprostol acid (II) and tetranoralfaprostol acid (III). These two metabolites represented 87–88% of the urine metabolites (62–72% of the dose was excreted in urine by 48 h). Tetranoralfaprostol acid converts to the δ-lactone (IV) under acidic conditions. The remaining excreted radioactivity consisted of several minor metabolites.

INTRODUCTION

In 1972 the prostaglandin PGF $_{2\alpha}$ was reported to cause regression of the corpus luteum (luteolytic effect) in cattle (Rowson et al.). A number of compounds in this class have been used in recent years to induce estrus (heat), with subsequent ovulation, by shortening the life span of the corpus luteum. Controlling estrus with these compounds provided a highly effective means for timing insemination, and therefore, the use of artificial insemination has become a much more useful reproduction management aid.

Alfaprostol (18,19,20-trinor-17-cyclohexyl-13,14-didehydro-PGF$_{2\alpha}$ methyl ester) (Figure 1), a compound with greater stability and selectivity than PGF$_{2\alpha}$, has been shown to be a potent luteolytic agent for use in inducing estrus in cows (Maffeo et al., 1983) and mares (Howey et al., 1983). Intramuscular administration of alfaprostol in cows shortened the life span of the corpus luteum and induced estrus within 75–96 h.

The major metabolic pathways that have been identified for natural prostaglandins are (a) oxidation of the 15-hydroxy group, (b) reduction of the Δ^{13} double bond, (c) β-oxidation of the carboxylic acid side chain to yield dinor and tetranor derivatives, and (d) ω-oxidation to produce hydroxy compounds and dicarboxylic acids (Bourne, 1979). Tetranor analogues have been found to be in a pH-dependent equilibrium with the corresponding δ-lactone (Brash, 1980; Brash, 1982; Bourne et al., 1980).

This report describes studies conducted to isolate and identify the major radiolabeled metabolites formed from alfaprostol in the cow. The metabolites distributed in selected tissues and urine were identified. In addition, metabolism of alfaprostol by the 9000 *g* bovine liver supernatant fraction was investigated.

Department of Animal Science Research, Hoffmann-La Roche Inc., Nutly, New Jersey 07110.

MATERIALS AND METHODS

Chemicals and Tissues. Unlabeled alfaprostol and unlabeled alfaprostol acid were obtained from Vetem SpA, Milano, Italy.

The compound 15-[^{14}C]alfaprostol was synthesized at Research Triangle Institute (RTI), Research Triangle Park, NC (Jeffcoat and Cook, 1979). The radiochemical purity was greater than 95%, and the specific activity was 132.0 μCi/mg. Its identity was established by TLC, GC, HPLC, and MS and its radiochemical purity by TLC and HPLC.

The 15-[^{14}C]alfaprostol acid was prepared by saponification of 15-[^{14}C]alfaprostol in 0.1 N aqueous KOH at 90 °C for 30 min. After cooling, the solution was washed three times with 2 volumes of hexane. The pH was adjusted to 3.5 with HCl and the resultant mixture extracted three times with 2 volumes of ethyl ether. The ether solution was stored at 4 °C.

Injection site muscle and urine from young adult holstein cows (400–600 kg of body weight) dosed with approximately 1.5 mg/100 kg of 15-[^{14}C]alfaprostol were obtained from the gross distribution studies conducted at RTI (Jeffcoat and Cook, 1981). Urine collections were made between 0 and 120 h. The 16–24-h urine was used for metabolite isolation because it contained the highest concentration of radioactivity.

The 16–24-h urine containing unlabeled metabolites of alfaprostol was obtained from a separate experiment in which one holstein cow was dosed with unlabeled alfaprostol in the same manner as the radiolabeled study described above.

0021-8561/86/1434-0688$01.50/0