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Effect of Ethylene on the Endogenous Cytokinin and Gibberellin Levels in Tuberizing Potatoes[1]

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ABSTRACT

High concentrations of 2-chloroethylphosphonic acid inhibited tuberization on aged potato tubers (*Solanum tuberosum*) that had been predisposed to the little tuber disorder. As a result of this treatment sprouts developed which contained relatively high levels of endogenous gibberellins and which elongated normally. The endogenous cytokinin levels in the different treatments did not change appreciably. It is suggested that tuberization is prevented by ethylene either as a direct inhibition of cell division or that it prevents the endogenous cytokinins from functioning. Irrespective of the mode of action of ethylene, cell division apparently is the primary process affected, the result being that storage tissue required for the accumulation of starch is not formed.

Courduroux (7) suggested that the specific tuber-forming stimulus (10) responsible for tuberization may be related to a cytokinin. Applied cytokinins have been shown to induce tuber formation and starch accumulation in *in vitro* grown potato stolons (17, 19). Further evidence for the participation of cytokinins in the tuberizing process is provided by the fact that stolon tips and small bulking potatoes contain high levels of endogenous cytokinins (22, 23).

Other hormones have also been implicated in tuberization. Gibberellins inhibit tuber formation (15) and have been shown to have no effect on starch accumulation in stolons (19). They do, however, promote stolon elongation (15). Natural tuberization (12) as well as the kinetin-stimulated accumulation of starch in stolons (13) are inhibited by ethylene. Ethylene also inhibits stolon elongation and causes the development of abnormal subapical swellings (13) devoid of any appreciable starch (6).

This investigation reports on the effect of ethylene on the levels of endogenous cytokinins and gibberellins in naturally tuberizing potatoes.

MATERIALS AND METHODS

Potato tubers (*Solanum tuberosum* L. cv. Up-to-date) were stored at 3 C in the dark for 10 months. After transferring these tubers to 20 C, premature tuberization occurred within 10 days. This phenomenon is known as the "little tuber" disorder (9, 22). If, however, the stored tubers are dipped for 5 min in solutions of 2-chloroethylphosphonic acid) (Ethrel) before being transferred to the higher temperature, tuberization is inhibited and sprouting occurs. Little tubers, sprouts, and mother tubers were collected 30 days after transferring the control and Ethrel-treated tubers to 20 C. This material was deep frozen (−20 C) and subsequently analyzed for cytokinins and gibberellins.

Extraction and Bioassay for Cytokinins. The frozen plant material (400 g) was homogenized with sufficient ethanol to give a final concentration of 80% ethanol. Cytokinins were extracted by means of a cation exchange resin (22) and assayed with the soybean bioassay (11). Acidic gibberellin-like compounds were extracted with ethyl acetate as previously described (5) and their levels determined with the *Rumex* leaf senescence bioassay (24). All extracts were strip-loaded onto Whatman No. 1 chromatography paper and separated with isopropyl alcohol-25% ammonium hydroxide-water (10:1:1, v/v). Dried chromatograms were divided into 10 equal R_F strips and each of these individually assayed for gibberellin or cytokinin activity.

RESULTS AND DISCUSSION

All experiments were carried out with tubers which had been predisposed to the formation of small daughter tubers (little tubers) and which did not produce any shoots (Fig. 1A). Treatment of these tubers with 1,000 and 5,000 mg/l Ethrel, before being transferred to 20 C, completely inhibited little tuber formation and resulted in the development of sprouts (Fig. 1, B and C). Morphologically the sprouts that developed were not all similar. At 1,000 mg/l Ethrel (Fig. 1B) many thin sprouts developed while at 5,000 mg/l Ethrel (Fig. 1C) a smaller number of thicker sprouts were formed.

As previously reported (22, 23), the developing little tubers contained higher levels of endogenous cytokinin than the mother tubers on which they formed (Figs. 2 and 3). These endogenous cytokinins co-chromatographed with zeatin and zeatin riboside. A comparison of the cytokinin levels in the control and Ethrel-treated potatoes showed that the activity of the sprouts was lower than that of the little tubers on the control potatoes while that of the mother tubers of the Ethrel-treated potatoes was slightly higher. The total cytokinin content of the different systems analyzed did, however, not differ appreciably (Fig. 3). This suggests that the morphological changes induced by Ethrel are not mediated by altering the levels of endogenous cytokinins.

The mother tubers, little tubers, and sprout material analyzed contained only one peak of acidic gibberellin-like activity (Fig. 4). This peak occurred at R_F 0.3 to 0.6 which is in agreement with previous reports (4, 8). The gibberellin-like activity in the mother tubers was low (Fig. 3). In the sprouts that developed as a result of Ethrel treatment the activity was higher than in the little tubers. This is in agreement with reports that gibberellin levels are high in actively growing sprouts (4, 20).

The inhibiting effect of Ethrel on tuberization could have been brought about in a number of ways. The level of gibberellins which is known to be high during extension growth before tuberization and which inhibits tuber formation (4, 15, 20, 21) could have been maintained by ethylene. This would explain the development of vigorously growing sprouts with high gibberellin activity (Fig. 3) on treated potatoes. As the endogenous level of cytokinins did not change in the Ethrel-treated tubers, ethylene

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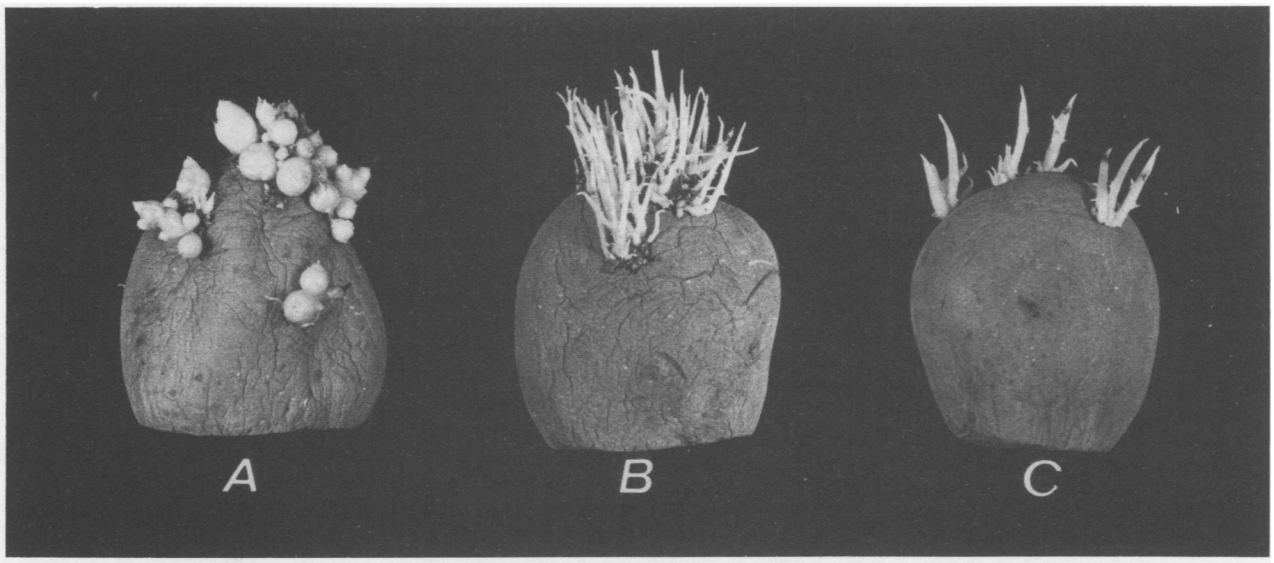

FIG. 1. Effect of Ethrel on the morphology of aged potato tubers (cv. Up-to-date) after 30 days at 20 C in the dark. A: control; B: 1,000 mg/l Ethrel; C: 5,000 mg/l Ethrel.

could also have prevented these compounds from acting at the subapical meristem, particularly as kinetin-induced tuberization (17, 19) can be reversed by this hormone (12, 13). This reversal could have been the result of an inhibition of cell division and/or starch synthesis. Both of these processes are stimulated by cytokinins (3, 14). Kinetin-induced starch accumulation at stolon tips has been shown to be the result of enhanced phosphorylase and pyrophosphorylase activity (14, 16). The effect of ethylene on these enzymes is not known. In the case of cell division, ethylene rapidly reduces mitosis in meristems and has been suggested to be a natural regulator of meristematic activity (1, 3). The possibility that ethylene inhibits tuberization by preventing cell division was thus investigated.

An anatomical and ultrastructural examination was made of the subapical meristems of dark-grown normal sprouts and stolons, sprouts that developed as a result of Ethrel treatment, and little tubers.

As reported earlier (18), stolons, before circumferential enlargement, exhibit the same morphological and anatomical features as apical buds. Ultrastructurally the same was true, suggesting that some physiological, probably hormonal, balance determines whether a shoot develops into a leafy sprout or a bulking tuber. All of the material examined contained starch indicating that ethylene does not eliminate its synthesis. Significant, however, was the fact that in the sprouts that developed as a result of Ethrel treatment cell division in the procambium was inhibited. In the swelling stolons this was not so and a large amount of unspecialized parenchyma developed from the procambium. This increase in the number of cells occurs during normal tuberization (2, 18) where cell division precedes starch accumulation (2).

From the available evidence, ethylene apparently prevents tuberization by eliminating or overriding the action of the cyto-

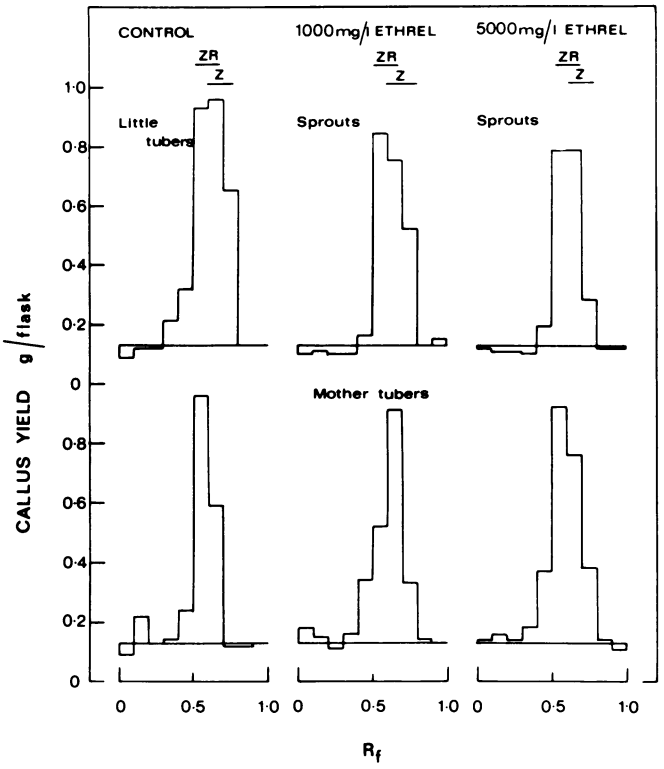

FIG. 2. Soybean bioassays of extracts obtained from 25 g equivalent of mother tuber, little tuber, and sprout tissue (cv. Up-to-date) after treating the aged tubers with Ethrel. The results presented are the means for two separate bioassays. Z: zeatin; ZR: zeatin riboside. Twenty-five μg/l kinetin gave a callus yield of 1.84 g/flask.

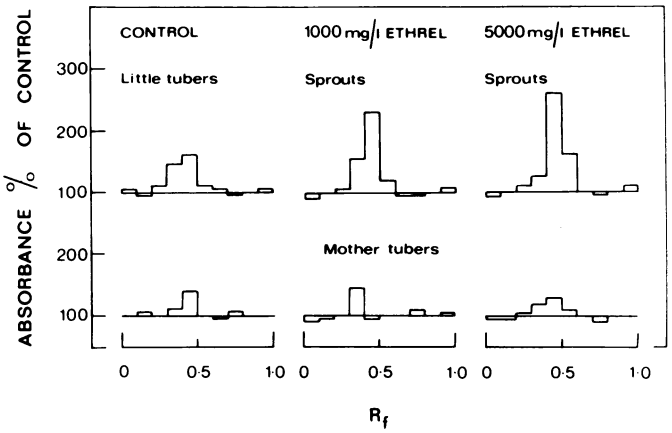

FIG. 4. *Rumex* leaf senescence bioassays of the acidic gibberellin-like substances obtained from 100 g equivalent of mother tuber, little tuber, and sprout tissue (cv. Up-to-date). Results presented are the means for two separate bioassays.

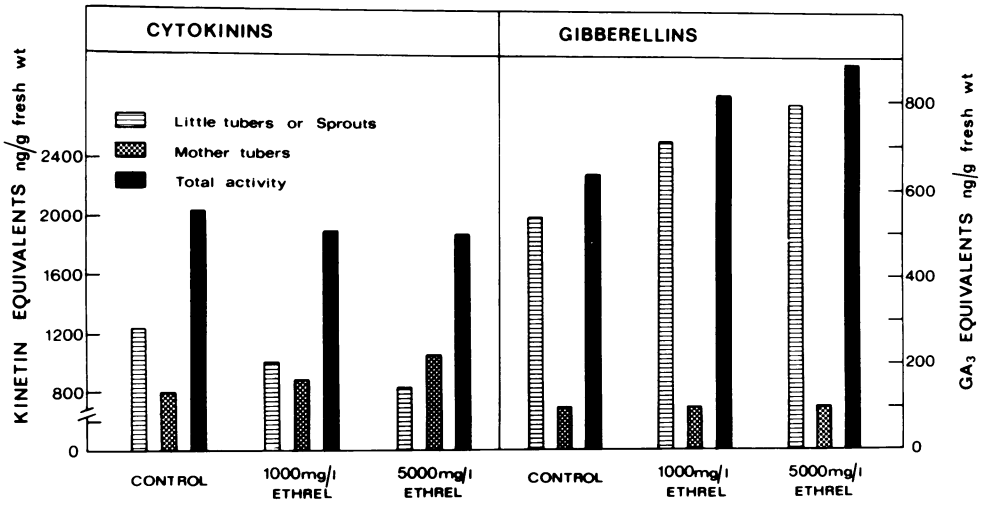

FIG. 3. Effect of Ethrel on the levels of cytokinins and acidic gibberellin-like substances in the mother tubers, little tubers, and sprouts of aged 'Up-to-date' potatoes. Results presented are the means for two separate bioassays.

kinins thus inhibiting cell division. As a result little storage parenchyma in which starch can be accumulated is formed. If the storage tissue is limiting it would be natural to expect that the concentration of the enzymes involved in starch synthesis will be low or undetectable.

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