Role of Ethylene in Naphthalene-Mediated Sprout Growth Inhibition in Potato

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Abstract
A number of substituted naphthalenes reversibly inhibit potato (Solanum tuberosum L.) sprout growth and may have potential as commercial sprout inhibitors. Sprout growth is also reversibly inhibited by ethylene treatment and ethylene production is often stimulated by xenobiotics. The role of endogenous ethylene in naphthalene-mediated sprout growth inhibition was examined using a variety of ethylene biosynthesis and action inhibitors. Ethylene evolution from intact potato tubers was significantly stimulated following treatment with alpha-naphthalene acetic acid (NAA), but not by 1,4- or 1,6-dimethylnaphthalene treatment. An increase in ethylene production was observed 24 h after NAA treatment, reached a maximum 48-72 h post-treatment and declined gradually thereafter. In addition to NAA, a variety of structurally unrelated auxins also stimulated ethylene production and inhibited sprout growth. Neither the non-competitive (Ag thiosulfate) nor the competitive (2,5-norbornadiene) ethylene antagonists affected NAA-induced growth inhibition. Simultaneous treatment with the ethylene biosynthesis inhibitor AVG blocked NAA-induced ethylene production but did not affect subsequent sprout growth inhibition. These results suggest that the sprout inhibiting effects of NAA, but not those of 1,4 or 1,6-dimethyl-naphthalene, are related to intrinsic auxin-like bioactivity and are not dependent on endogenous ethylene synthesis or action.

INTRODUCTION
Maintenance of postharvest potato tuber quality is of utmost concern to producers, processors, and consumers alike. Failure to maintain product quality can result in commercially unacceptable product for the processor and financial ruin for the producer. Postharvest quality losses in stored potatoes can occur through both physiological and disease-related processes. Of the physiological processes affecting potato storage and market quality, tuber dormancy and sprouting is one of the most important. Uncontrolled postharvest sprouting results in increased levels of toxic glycolalkaloids, increased respiration and transpiration, accelerated starch breakdown with concomitant accumulation of undesirable reducing sugars and decreased vitamin content. All of these changes adversely affect potato market quality and nutritional value resulting in lower producer prices and a nutritionally inferior product.

In the U.S., postharvest sprouting of potatoes is controlled primarily by one of two methods: preharvest treatment with maleic hydrazide (MH) or postharvest applications of chlorpropham (CIPC). Concern over the use of synthetic chemistries has prompted the search for alternative sprout control agents. Originally identified as a natural constituent of potatoes (Meigh et al., 1973), 1,4-dimethylnaphthalene has been commercially introduced as a postharvest sprout control agent (1,4 Insight). Other dimethylnaphthalene (DMN) isomers are also effective sprout inhibitors (Beveridge et al., 1981; Lewis and Shetty, 1997). In addition, early studies conducted at the Boyce Thompson Institute demonstrated that α-naphthalene acetic acid (NAA) treatment inhibited sprout growth (Guthrie, 1939). In spite of their effectiveness, to date their mechanism(s) of action remain uncharacterized. This ignorance hinders the systematic improvement of their efficacy as well as the identification of other, possibly more efficacious, sprout inhibitors.
Ethylene has long been known to be an effective and reversible inhibitor of potato sprout growth (Elmer, 1932; Rylski et al., 1974). Ethylene production can be stimulated by a variety of hormonal and non-hormonal agents including many naphthalene derivatives (Abeles et al., 1992). In this presentation, the effects of sprout-inhibiting naphthalenes on endogenous ethylene evolution are described and the physiological role of endogenously produced ethylene in naphthalene-mediated sprout growth inhibition is assessed.

MATERIALS AND METHODS

Certified, greenhouse-grown and disease free potato (Solanum tuberosum L. cv. ‘Russet Burbank’) minitubers (3-5 g tuber\(^1\)) were obtained from a commercial grower (Valley Tissue Culture, Halstad, MN, USA). Following harvest, tubers were stored at 3°C in a seed potato storage facility under ambient (ca. 90%) relative humidity. Tubers were used after a minimum of 6 months of storage when dormancy had naturally ended. Tubers were transferred to 20°C for 3 days prior to experimental use.

All compounds tested for sprout inhibition were initially dissolved in DMSO as 1000x concentrates. 1,4-DMN, 1,6-DMN and \(\alpha\)-NAA (NAA) were obtained from Sigma-Aldrich, St. Louis, MO, USA. Tubers (10/treatment) were treated by partial immersion in treatment solution (containing 1 drop Tween-20/50 mL) on an oscillating shaker (100 rpm) for 4 hours. Control tubers were treated as above with 0.1% (v/v) aqueous DMSO - Tween-20 alone. After treatment, tubers were allowed to air dry and were incubated in the dark at 20°C (>90% RH). The length of the longest sprout on each tuber was determined after 2 weeks.

For the determination of ethylene production, 3 treated tubers were sealed in plastic syringes (40 mL volume) and incubated in the dark (20°C) for 8 hours. The ethylene content of the syringe headspace was determined by GC using an alumina column. Tubers were treated with silver thiosulfate (STS, 4 mM Ag\(^+\)) for 1 hour by partial immersion (as above) immediately after water or naphthalene treatment. The effects of aminoethoxyvinylglycine (AVG, Fluka Chemical Co.) on naphthalene-induced ethylene production and sprout inhibition were examined by including AVG (0.1mM) in the initial treatment solution. The effects of 2,5-norbornadiene (NBD, Sigma-Aldrich) were examined by enclosing control or treated tubers in 4.5 L plexiglas containers containing 10-40 uL liquid NBD on cotton wadding. The containers were opened, vented and fresh NBD added after 7 days.

RESULTS

Ethylene evolution from control tubers was low and relatively constant for four days after the start of the experiment (Fig. 1A). The onset of visible sprout growth (after 4 days) was accompanied by a sustained increase in ethylene production. One day after treatment with 1 mM 1,4- or 1,6-DMN, ethylene production was slightly stimulated (\(< 2\)-fold), returned to control levels by day 2, and remained at or below control values thereafter. NAA treatment resulted in a rapid and sustained increase in ethylene production. One day after treatment, ethylene production in NAA-treated tubers was ca. 3.5x control and it increased further over the next three days reaching a maximum of nearly 19x control 3 days after treatment. By day 7, ethylene production in NAA-treated tubers was below that of controls and remained depressed for the duration of the experiment.

At concentrations of 1 µM or higher, NAA stimulated ethylene production in a dose-dependent manner with treated tubers exhibiting ca. 3-fold, 6-fold, 9-fold and 19-fold increases at 1, 10, 100 and 1000 µM, respectively (Fig. 1B). In all cases, maximum stimulation of ethylene production was observed 2-3 days post-treatment, declining rapidly thereafter (data not presented). Two weeks after treatment, sprout growth was inhibited by NAA at concentrations of 100 µM or higher with 1 mM causing near-total inhibition. Even at the highest NAA concentrations, growth inhibition was transient and by 4 weeks after treatment, active sprout growth was observed in treated tubers. In addition to NAA, other auxin-like agonists stimulated ethylene production and inhibited
sprout growth while inactive analogs such as -NAA were ineffective (data not presented).

The role of ethylene in NAA-mediated sprout inhibition was determined using inhibitors of both ethylene biosynthesis and action. In the absence of NAA, AVG had no effect on the basal rate of ethylene production (Fig. 2A). As before, NAA treatment resulted in a large (ca. 50-fold) increase in ethylene production 3 days post-treatment. Simultaneous treatment with 0.1 mM AVG, inhibited NAA-induced ethylene production by 80%. AVG was not toxic to potato tubers and AVG treatment had no effect on sprout growth in untreated tubers. Sprout growth was largely inhibited by NAA treatment and AVG did not reverse this inhibition.

Treatment of control tubers with the non-competitive ethylene antagonist silver thiosulfate (STS) had no effect on sprout growth (Fig. 2B). NAA treatment inhibited sprout growth by 90%. STS treatment had no effect on NAA-induced sprout growth inhibition. Continuous exposure to increasing concentrations of the competitive ethylene antagonist NBD had no effect on NAA-induced sprout growth inhibition (Fig. 2C).

DISCUSSION

The sprout growth inhibiting activities of NAA and certain DMN isomers have been known for some time (Guthrie, 1939; Meigh et al., 1973). In spite of this, little is known regarding their mechanisms of action in potato sprouts. Of the two types of naphthalene derivatives, only NAA displayed consistent sprout inhibitory activity. Under the conditions used in these studies, both 1,4- and 1,6-DMN isomers elicited inconsistent and weak inhibitory activity. The underlying basis(es) for this inconsistent performance is unclear but may relate to the fact that pure isomers (as opposed to complex mixtures) were used in the present studies.

Ethylene production can be stimulated by a variety of hormonal, non-hormonal and environmental factors (Abeles et al., 1992). In non-aquatic angiosperms, exogenous ethylene typically inhibits longitudinal growth in both roots and shoots. Potato tuber sprouts are etiolated shoots and exhibit typical ethylene responsiveness. As such, a role for ethylene in chemically induced growth inhibition should always be considered.

In general, ethylene production by both control and treated tubers was very low and never exceeded 100 pL/h/ g fresh weight. Both 1,4- and 1,6-DMN treatment elicited a small (< 2-fold) and transient increase in ethylene production that returned to basal levels within 48 hours following treatment (Fig. 1A). In contrast, NAA treatment elicited a much larger (up to 50-fold) increase in ethylene production that persisted for at least 4 days following treatment. However, this stimulation was also transient and ethylene production was at or below control levels 7 days after treatment. Ethylene production can be stimulated by a variety of factors including stress and hormones (Abeles et al., 1992). Given the magnitude and duration of ethylene stimulation in these tubers, it is likely that the DMN isomers elicited a stress response while NAA acted hormonally. Consistent with this scenario, other auxinic agonists stimulated ethylene production and inhibited sprout growth in potatoes (unpublished data).

Ethylene is a potent inhibitor of sprout growth in potatoes with an IC50 concentration of 0.1 ppm (Suttle, unpublished data). Although effective in this regard, sprout growth resumes almost immediately upon cessation of ethylene treatment (Rylski et al., 1974; Suttle, unpublished data). In NAA treated potatoes, elevated ethylene production persisted for less than 7 days after treatment (Fig. 1A) yet sprout growth was inhibited for up to 3-4 weeks (unpublished data). In addition, inhibitors of ethylene synthesis or action had no effects on NAA-induced sprout growth inhibition (Figs. 2A, B, & C). Taken as a whole, these data are not consistent with a role for ethylene in DMN or NAA-mediated sprout growth inhibition.

It is proposed that the two types of naphthalene derivatives inhibit potato sprout growth by distinct mechanisms, neither of which involve endogenous ethylene. The mechanism(s) of action of the DMN isomers is unresolved while that of NAA is related to its auxin-like activity.
Literature Cited
Guthrie, J.D. 1939. Inhibition of the growth of potato tubers with the vapor of the methyl ester naphthalene acetic acid. Contrib. Boyce Thompson Instit. 10: 325-328.
Fig. 1. A. Time-course of ethylene production from intact potato tubers following treatment with 1 mM naphthalene derivatives. Inset: length of longest sprout per tuber 14 days post-treatment. CON: control tubers; NAA: α-Naphthalene acetic acid; 1,4: 1,4-dimethylnaphthalene; 1,6: 1,6-dimethylnaphthalene.

Fig. 2. A. Effects of 0.1 mM AVG ± 1mM NAA on ethylene production 3 days post-treatment (solid bars), and length of longest sprout 14 days post-treatment (hatched bars). B. Effects of silver thiosulfate (STS) on ± 1mM NAA on sprout length 14 days post-treatment. C. Effects of increasing amounts of 2,5-norbornadiene (NBD) on sprout length in NAA-treated tubers 14 days post-treatment.