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Toxicity and Metabolism of Exogenous α,β-Unsaturated Carbonyls in Potato (*Solanum tuberosum* L.) Tubers

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ABSTRACT: A group of aliphatic α,β-unsaturated carbonyl compounds was evaluated for their utility as inhibitors of sprout growth in stored potato tubers (*Solanum tuberosum* L.). Nondormant tubers were treated with vapors of six 8−10-carbon compounds of this chemistry. Subsequent sprout growth at 16 °C (95% relative humidity) over ca. 3 months in storage was suppressed by all compounds in a concentration-dependent manner. The volatile metabolites produced by sprout and associated tuber tissues following treatment with 3-octen-2-one, 3-nonen-2-one, and 3-decen-2-one were the corresponding alkyl ketones and alkyl secondary alcohols. In contrast, (*E*)-2-octenal, (*E*)-2-nonenal, and (*E*)-2-decenal were metabolized by two pathways: (1) parent compound to the corresponding alkyl aldehyde and then to the alkyl primary alcohol and (2) parent compound to the alkenyl primary alcohol. Residues of 3-nonen-2-one and (*E*)-2-nonenal and their metabolites were analyzed in whole tubers over a 28 day post-treatment period. The concentrations of the parent ketone and aldehyde declined rapidly following application, and the most persistent metabolites were 2-nonanol and (*E*)-2-nonen-1-ol, respectively. The sequence of reactions leading from the α,β-unsaturated carbonyls to the alcohols was determined by application of each of the 9-carbon compounds individually to tubers. In long-term efficacy studies, a single application of (*E*)-2-nonenal and 3-nonen-2-one to nondormant tubers terminated sprout growth and prevented regrowth for 2−3 months. A second application suppressed sprouting for at least 4−5 additional months. This efficacy, combined with rapid metabolism and low residue levels, makes the 8−10-carbon α,β-unsaturated ketones and aldehydes worth consideration for use as sprout inhibitors.

KEYWORDS: *Solanum tuberosum, potato tubers, sprout growth, α,β-unsaturated carbonyl, aldehyde, ketone, sprout inhibition, metabolite, residue*

INTRODUCTION

α,β-Unsaturated aldehydes and ketones are ubiquitous in the environment. Some, such as the three-carbon aldehyde acrolein, are combustion byproducts, whereas many others in this chemical class occur naturally in plant and animal tissues. For example, 4-hydroxy-(*E*)-2-nonenal and (*E*)-2-hexenal are produced by normal cellular metabolism during the oxidation of unsaturated fatty acids.[1] α,β-Unsaturated ketones such as 3-nonen-2-one and 3-decen-2-one occur in higher plants, where they are components of the aroma profile in certain fruit and mushroom species.[2,3] These bifunctional molecules, containing a carbonyl and a conjugated double bond, are soft electrophiles capable of forming adducts with cellular sulfhydryl and amino groups such as those found in glutathione, proteins, and DNA.[1,4] Alterations to these essential biomolecules can be toxic, and thus cells must invoke defensive mechanisms to mitigate potential damage. In plants, the carbonyl groups and double bonds of these compounds are enzymatically converted to less reactive alcohols and single bonds, respectively.[5]

Despite these protective strategies, a threshold concentration of α,β-unsaturated carbonyls that is lethal to the organism can be reached. Toxicity data for α,β-unsaturated carbonyls is largely derived from mammalian, aquatic, and microbiological systems. For example, juvenile nematodes were rendered immotile by (*E*)-decenal and (*E*,*E*)-2,4-decadienal.[6] Antifungal activity of 6- and 9-carbon α,β-unsaturated aliphatic compounds toward *Alternaria* was described by Andersen et al.[7] Due to the generic nature of their damaging effects on cells, it is probable that with sufficient dosage, exogenous application of α,β-unsaturated carbonyls to higher plant organs will result in some level of tissue injury and may therefore have some utility as a herbicidal treatment. For example, Bradow and Connick[8] reported that vapors of (*E*)-2-hexenal inhibited seed germination in onion, tomato, and carrot.

Because of the potential for commercial application, we chose potato tuber (*Solanum tuberosum* L.) etiolated sprouts as our model system to evaluate the toxicity of α,β-unsaturated carbonyl compounds. Achieving effective sprout inhibition is a major challenge for long-term storage of potatoes. The prospect of using naturally occurring compounds, although not a guarantee of safety, is appealing to consumers concerned about health and environmental effects. The technology to fog volatile liquid compounds into large commercial potato storages is available. However, the feasibility of using such compounds will depend not only on their efficacy but also on the amount and nature of the residues left on and in tubers. The studies reported herein demonstrate the toxicity of 8-, 9-, and 10-carbon α,β-unsaturated aldehydes and ketones to potato sprouts, as well as their progressive metabolism in sprout and tuber tissues. Metabolites are identified, and tentative reaction

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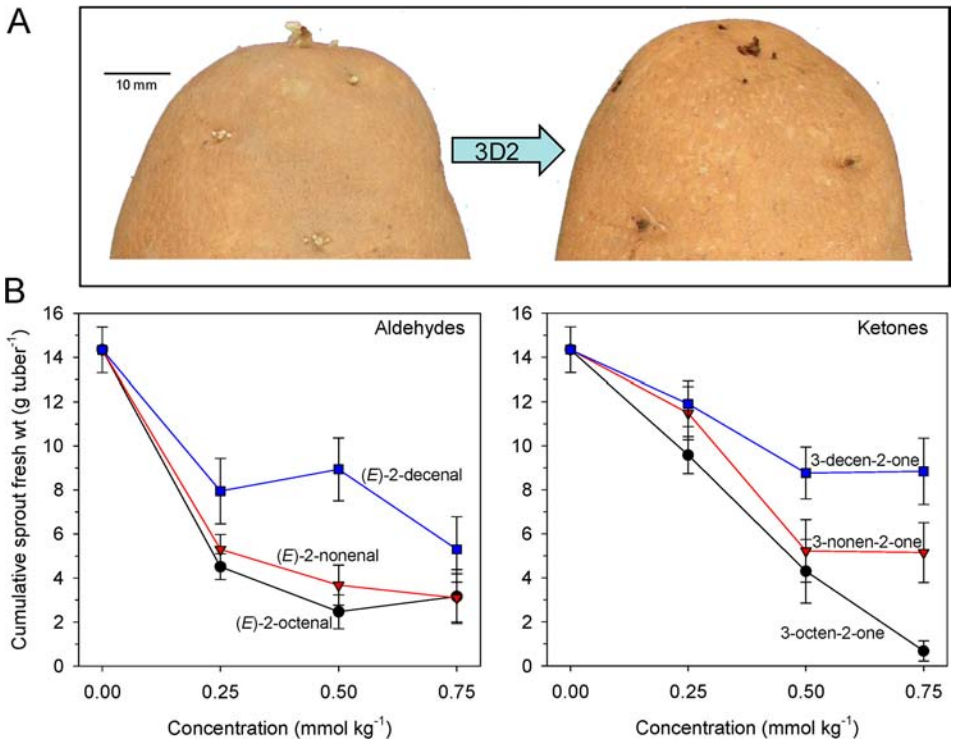

Figure 1. (A) Necrosis of etiolated sprouts in the apical eyes of tubers treated for 24 h with 3-decen-2-one (3D2) at 0.75 mmol kg^{-1} tuber fresh weight. Tubers were treated at the peeping stage of sprout development. (B) Sprout growth from 'Russet Burbank' tubers treated with four concentrations of six α,β-unsaturated aldehydes and ketones. Tubers were treated at room temperature for 24 h and then stored at 16 °C (95% RH) for 89 days following application. Each point is the mean cumulative sprout fresh weight from six tubers ± SE.

sequences based on precursor−product relationships of individual metabolites are presented.

MATERIALS AND METHODS

Chemicals. (*E*)-2-Octenal, (*E*)-2-nonenal, (*E*)-2-decenal, 3-octen-2-one, 3-nonen-2-one, 3-decen-2-one, and (*E*)-2-nonen-1-ol were obtained from Bedoukian Research Inc. (Danbury, CT, USA). Nonanal, 1-nonanol, 2-nonanol, and 2-nonanone were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant Material. 'Russet Burbank', 'Ranger Russet', and 'Umatilla Russet' tubers were grown at the Washington State University (WSU) Irrigated Agriculture Research and Extension Center at Othello, WA (46° 47.277′ N latitude, 119° 2.680′ W longitude) using standard cultural practices. Initial storage following harvest was at 12 °C (95% relative humidity (RH)) for 10 days to facilitate wound healing. Tubers were subsequently stored at 9 °C (95% RH) unless indicated otherwise. With the exception of brief periods for application of chemicals and harvesting of sprouts, tubers were kept in darkness, and all sprouts that developed were therefore etiolated.

Bioassay for Toxicity to Potato Sprouts. (*E*)-2-Octenal, (*E*)-2-nonenal, (*E*)-2-decenal, 3-octen-2-one, 3-nonen-2-one, and 3-decen-2-one were applied to 'Russet Burbank' tubers in a bioassay for toxicity to sprout tissue just beginning to emerge (i.e., peeping stage; sprouts 1−2 mm long as depicted in Figure 1A). Tubers were stored at 9 °C (95% RH) in the dark following harvest until they displayed small (≤2 mm) sprouts (peeping stage). For each treatment, six tubers with a total fresh weight of 731 ± 3 g were placed into a 3.9 L glass jar. The jar lids were lined first with aluminum foil and then filter paper. The volume of compound required to achieve 0, 0.25, 0.50, or 0.75 mmol kg^{-1} tuber fresh weight was pipetted onto the filter paper immediately before the jar was sealed. One jar of six tubers received no chemical and served as the nontreated control. The jars remained closed at room temperature in darkness for 24 h. Tubers were then removed from the jars and placed at 16 °C (95% RH) in darkness to sprout. The sprout fresh weight produced over an 89 day storage period was recorded as a measure of toxicity of the α,β-unsaturated aldehydes and ketones. The study was set up in a randomized complete block design with factorial arrangement of treatments (four concentrations of six compounds) and six replicates of single tubers.

Identification of Metabolites. Nondormant 'Ranger Russet' tubers were transferred from 4 °C storage to 22 °C (95% RH) in darkness to accelerate sprouting. Treatments began when sprouts measured at least 2 cm in length. 3-Octen-2-one, 3-nonen-2-one, 3-decen-2-one, (*E*)-2-octenal, (*E*)-2-nonenal, and (*E*)-2-decenal were each applied at 0.5 mmol kg^{-1} tuber fresh weight to three replicates of four tubers each (average total fresh weight = 742 ± 4 g) as described for the bioassay. Following a 24 h exposure, sprouts were removed from the four tubers, pooled, frozen in liquid N_2, and stored at −80 °C until analysis.

Identification of metabolites in sprouts treated with 3-nonen-2-one and (*E*)-2-nonenal was accomplished with gas chromatography−mass spectrometry (GC-MS). Samples for GC-MS were prepared by grinding frozen sprout tissue to a fine powder in a mortar with liquid N_2. One gram of frozen powder was transferred to a 10 mL reaction vial containing a stir bar, 1.8 g of NaCl, and 3.7 mL of H_2O. The vial was immediately closed with a septum. Following 15 min of stirring, a solid phase microextraction (SPME) fiber (PDMS/DVB, Sigma-Aldrich) was inserted through the septum cap into the headspace of the vial, and volatile compounds were collected for 45 min. An HP 5890 gas chromatograph fitted with a DB-1 capillary column (length = 60 m, i.d. = 0.32 mm, film thickness - 0.25 μm) was interfaced to an HP 5971 mass selective detector operated at 70 eV. The SPME fiber was desorbed for 3 min in a 200 °C inlet operated in splitless mode. The helium flow rate was 1.0 mL min^{-1}, and the oven was programmed as follows: 35 °C for 3 min, 2 °C min^{-1} to 50 °C, 5 °C min^{-1} to 225 °C; hold for 5 min. Spectra from samples were compared to those of authentic standards and a Wiley-NBS library to confirm the identity of peaks unique to treated tissue. Solutions (10000 ppm) of these identified compounds and their 8- and 10-carbon analogues were prepared in acetonitrile. These solutions served as retention time markers and single-point calibration standards for the

compounds in the GC-FID analysis of sprout tissue from the six treatments. The standards were analyzed by adding 1 μL to 1 g of frozen, nontreated sprout tissue in prepared vials with volatile collection as described above. Three replicates of each of the six chemical treatments plus nontreated controls were analyzed by GC-FID. For these analyses the SPME fiber was desorbed for 3 min at 200 °C in the inlet (split 5:1) of an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with the same column as the GC-MS and a flame ionization detector operated at 275 °C. The helium carrier gas flow was 1.6 mL min^{-1}. The oven was programmed at 8 °C min^{-1} from 33 to 225 °C. Compounds of interest were quantified via the response factors obtained from the analysis of tissue spiked with authentic standards.

Metabolic Pathway Study. A study was designed to elucidate the precursor−product relationships in the metabolism of 3-nonen-2-one and (*E*)-2-nonenal using the glass jar bioassay system. 'Ranger Russet' tubers with 2 cm long sprouts (four tubers per treatment jar; total tuber fresh weight = 1020 ± 5 g) were treated with separate applications of 3-nonen-2-one and its two metabolites, 2-nonanone and 2-nonanol, each at four concentrations, 0.75, 0.50, 0.25, and 0.125 mmol kg^{-1} tuber fresh weight. A separate study used 'Russet Burbank' tubers (four per treatment jar, 888 ± 10 g) and applied (*E*)-2-nonenal and its metabolites, nonanal, (*E*)-2-nonen-1-ol, and 1-nonanol, at each concentration. Twenty-four hours following treatment, a cork borer (15 mm diameter) was used to remove cylindrical sections of tissue centered over a sprout, taking seven samples from each tuber. The outer 3 mm of these cylinders, with sprouts attached, were frozen in liquid N_2 and stored at −80 °C until analysis. The treatment compounds and their metabolites were collected from two randomly chosen disks from each tuber (four replicates) using a SPME fiber and analyzed as described in the preceding section. Quantitation was accomplished through the use of multilevel calibration using authentic standards added to nontreated tissue.

Time-Dependent Residue Studies. The persistence of 3-nonen-2-one, (*E*)-2-nonenal, and their respective metabolites was followed for 28 days in 'Ranger Russet' tubers. Each compound was applied to tubers in 190 L plastic barrels. Barrels were fitted with a 15 cm fan centered in the bottom, which was shielded by a cylindrical wire cage that extended upward through the central part of the chamber. Tubers (∼22 kg total fresh weight) in mesh bags were stacked around the cage along the outer circumference of the barrel, and a piece of filter paper was placed atop the cage so as not to contact the tubers. The appropriate volume of test compound to achieve 0.75 mmol kg^{-1} tuber fresh weight was pipetted onto the filter paper, and the barrels were sealed. The fan was turned on, and the barrels remained closed at room temperature for 24 h. Tubers were stored at 9 °C (95% RH) in the dark following treatment. Nontreated tubers (also enclosed in barrels) were used for background controls and as a matrix for the calibration standards. Tuber tissue samples for residue analyses were taken prior to treatment, immediately after barrel opening, and at 1, 2, 3, 7, 14, 21, and 28 days of storage. A 3−4 mm central slice was removed along the apical-basal axis of the tuber and chopped finely before being frozen in liquid N_2 and stored at −80 °C until the last (28 day) sample was processed. Three replications were used; each replicate contained the pooled tissue from four tubers. Analysis of 2 g of treated tissue was via SPME and GC-FID as described for the identification of metabolites. Quantitation of all compounds was via multilevel calibration with external standards added to nontreated tissue.

Duration of Sprout Suppression. A long-term efficacy study using (*E*)-2-nonenal and 3-nonen-2-one for sprout suppression of three cultivars was completed during the 2003−2004 storage season. 'Ranger Russet' (RR), 'Umatilla Russet' (UR), and 'Russet Burbank' (RB) tubers were grown at the WSU Research Unit (Othello, WA, USA) and harvested on September 29 (UR and RB) and October 1, 2003 (RR). After a brief (10 day) wound-healing period at 12 °C (95% RH), the tubers were stored at 9 °C (95% RH) in the dark until treatment. The compounds were initially applied to all tubers of a specific cultivar as they emerged from dormancy at the peeping stage of sprout development (sprouts ≤2 mm long). A subsample of tubers was then retreated when sprout growth resumed. 'Russet Burbank' and 'Umatilla Russet' tubers were treated on February 11 and April 18, 2004, 135 and 202 days after harvest (DAH), respectively. 'Ranger Russet' tubers were treated on January 13 and March 22, 104 and 173 DAH, respectively. Both compounds were applied individually to approximately 30 kg of tubers at a rate of 0.75 mmol kg^{-1} tuber fresh weight in 190 L plastic barrels as described in the preceding section. Following treatment, tubers were stored in the dark at 9 °C (95% RH). A sample of 12 tubers (replicates) was evaluated monthly for sprout fresh weight. Sprout fresh weight data were plotted versus DAH.

Data Analysis and Presentation. Sprout growth (fresh weight), sprout metabolite concentration, and tuber residue level data were subjected to analysis of variance. Treatments (carbonyl compound, rate, storage duration) were arranged factorially in randomized complete block designs for all studies. When appropriate, sums of squares were partitioned into main effects and interactions, and significance levels (*P* values) are reported. Data are plotted ± SE. Results from all studies were confirmed at least once in studies identical or similar to those presented herein.

RESULTS

Bioassay for Toxicity to Potato Sprouts. Six α,β-unsaturated carbonyl compounds were evaluated for toxicity to emerging potato sprouts in a small-scale bioassay. All chemical treatments induced rapid necrosis of sprout tissue during 24 h of exposure to the vapors at room temperature, as evidenced by blackening (necrosis) of the buds (meristems) in the eyes (nodes) of tubers (Figure 1A). Figure 1B shows the cumulative fresh weight of sprouts produced by tubers over 89

Table 1. Compounds Recovered from 'Ranger Russet' Sprouts following a 24 h Exposure to α,β-Unsaturated Aldehydes and Ketones Applied at 0.50 mmol kg^{-1} Tuber Fresh Weight[a]

compd applied	compd recovered	concn (nmol g^{-1} fresh wt)
3-octen-2-one	2-octanone	168.1 ± 10.4
	2-octanol	153.6 ± 4.0
	3-octen-2-one	13.6 ± 4.1
3-nonen-2-one	2-nonanone	285.3 ± 15.4
	2-nonanol	107.0 ± 14.6
	3-nonen-2-one	70.9 ± 17.3
3-decen-2-one	2-decanone	219.3 ± 15.8
	2-decanol	165.7 ± 14.6
	3-decen-2-one	58.5 ± 17.9
(*E*)-2-octenal	octanal	9.4 ± 0.6
	(*E*)-2-octen-1-ol	91.4 ± 5.1
	1-octanol	107.0 ± 6.0
	(*E*)-2-octenal	50.6 ± 4.9
(*E*)-2-nonenal	nonanal	0.4 ± 0.1
	(*E*)-2-nonen-1-ol	51.9 ± 10.2
	1-nonanol	32.3 ± 9.9
	(*E*)-2-nonenal	53.1 ± 30.0
(*E*)-2-decenal	decanal	0.7 ± 0.2
	(*E*)-2-decen-1-ol	110.4 ± 10.5
	1-decanol	24.3 ± 4.2
	(*E*)-2-decenal	94.8 ± 33.6

[a]Data are the average of three replicates ± standard error.

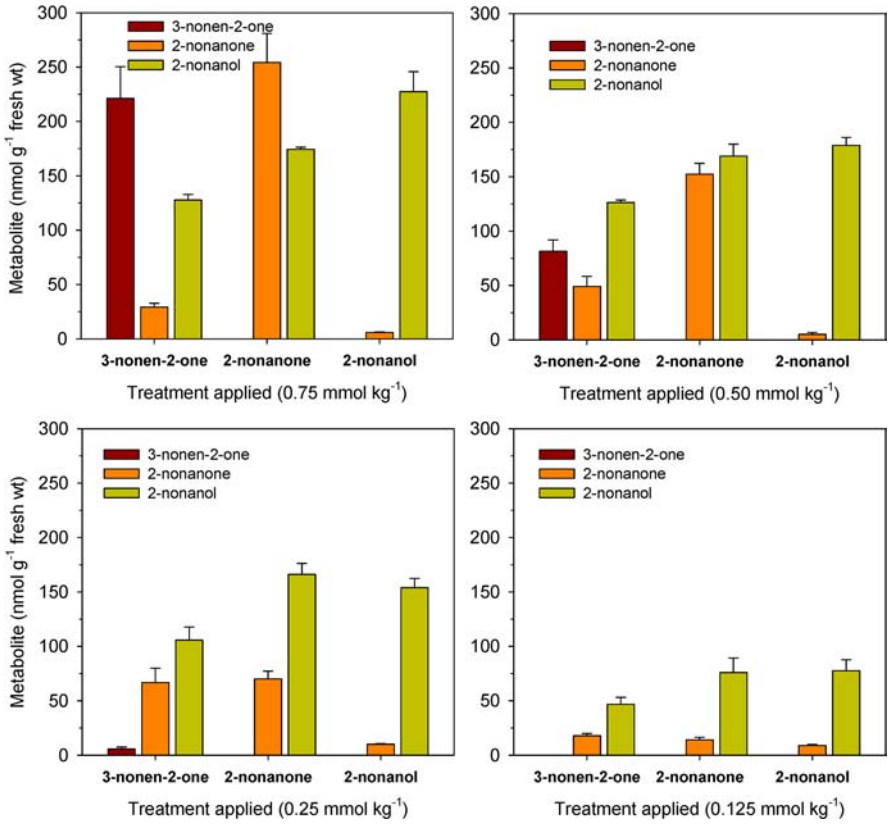

Figure 2. Metabolites recovered from sprout and associated tuber tissue following 24 h of treatment of 'Ranger Russet' tubers with 3-nonen-2-one, 2-nonanone, or 2-nonanol at four concentrations as specified in the *x*-axis labels. Sprouts were ca. 2 cm long at the time of treatment. Data are means of 8 sprout/tissue disks randomly chosen from 28 to represent four tubers. Vertical bars are SE.

days of storage at 16 °C in response to an initial 24 h treatment with each compound at four concentrations. The inhibition of subsequent sprout growth was dose-dependent ($P < 0.001$), and the extent of inhibition depended on the carbonyl compound (compound × rate, $P < 0.001$). At 0.75 mmol kg^{-1} tuber fresh weight, 3-octen-2-one, (*E*)-2-octenal, and (*E*)-2-nonenal inhibited sprout growth most effectively, resulting in 97, 78, and 78% inhibition, respectively, when compared with nontreated tubers. The half-maximal inhibitory concentrations (IC_{50}, mmol kg^{-1}) were estimated from the first- or second-degree polynomials describing the relationships (Figure 1B). In descending order of effectiveness were (*E*)-2-octenal (IC_{50} = 0.18), (*E*)-2-nonenal (IC_{50} = 0.21), 3-octen-2-one (IC_{50} = 0.37), 3-nonen-2-one (IC_{50} = 0.45, (*E*)-2-decenal (IC_{50} = 0.50), and 3-decen-2-one (IC_{50} = not reached). As a group, the aldehydes were more effective in sprout suppression than the ketones (aldehyde vs ketone, $P < 0.001$).

Metabolites of α,β-Unsaturated Carbonyls. Application of six α,β-unsaturated carbonyls to sprouting potato tubers was followed by extraction and analysis of volatile compounds from the sprout tissue to determine the presence of any compounds in treated sprouts that may have arisen from conversion of the chemical applied. None of the compounds in Table 1 were detected in sprouts from nontreated tubers. A sublethal dose of 0.5 mmol kg^{-1} tuber fresh weight was selected to avoid excessive sprout tissue mortality and allow at least some metabolism to occur. Twenty-four hours following treatment with α,β-unsaturated ketone vapors, sprouts contained two previously undetected volatile species in addition to the original compound (Table 1). In all cases, the saturated ketones and saturated secondary alcohols corresponding to the parent compounds were detected in concentrations greater than that of the parent. Regardless of the α,β-unsaturated ketone applied, the saturated ketone was the most abundant (49−62 mol %) of the three recovered species.

The compounds recovered from sprouts following treatment of tubers with α,β-unsaturated aldehydes were the saturated aldehydes, unsaturated primary alcohols, and saturated primary alcohols corresponding in carbon number to the parent compounds (Table 1). Octanal, nonanal, and decanal formed the smallest percentage of the total residue in their respective groups (0.3−3.6 mol %). For (*E*)-2-nonenal and (*E*)-2-decenal, the corresponding unsaturated primary alcohols were the most abundant species (38 and 48 mol %, respectively), whereas 1-octanol was the major component (41 mol %) of residue for (*E*)-2-octenal-treated sprouts.

Pathway of Metabolism. The results presented in Table 1 partly address the fate of α,β-unsaturated carbonyls applied to potato sprouts but do not illuminate the precursor−product relationships. To elucidate the sequence of the reactions leading from 3-nonen-2-one and (*E*)-2-nonenal to the various products, we applied the parent compounds and their previously identified metabolites individually at various rates to sprouting tubers and then extracted and analyzed the products from the treated tissue. Figure 2 summarizes the results for 3-nonen-2-one. Consistent with the preceding study, this 9-carbon α,β-unsaturated ketone was metabolized to 2-nonanone and 2-nonanol at each dosage. The lowest dosage (0.125 mmol kg^{-1}) resulted in 100% conversion of the parent compound. Higher rates apparently injured tissue sufficiently to inhibit complete

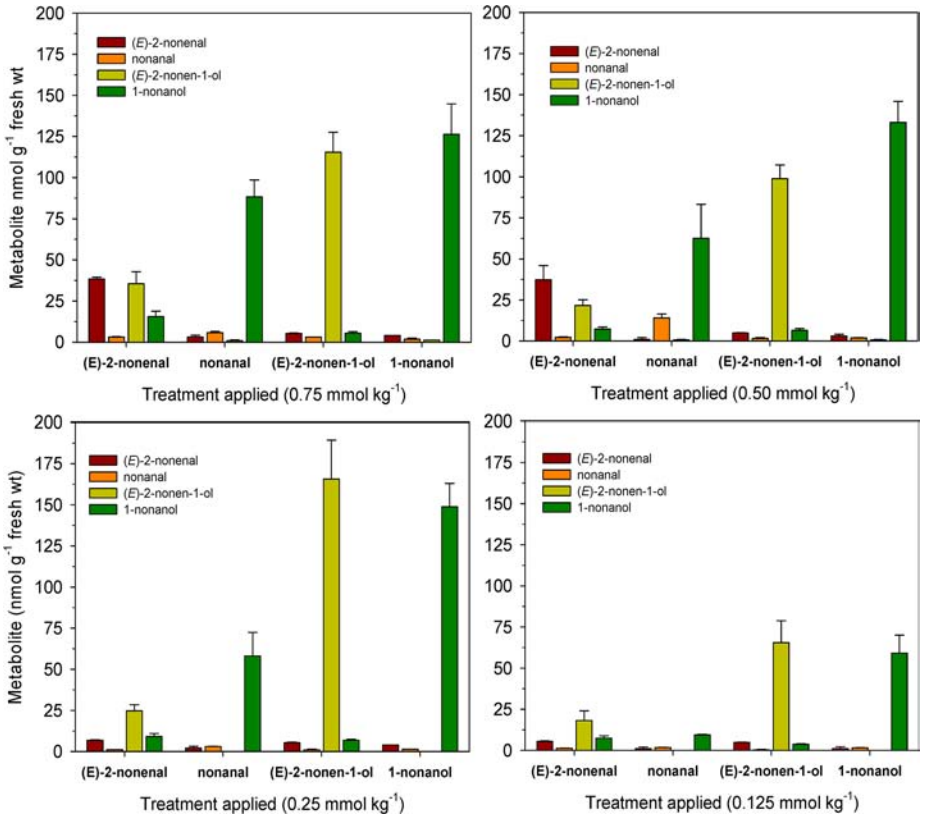

Figure 3. Metabolites recovered from sprout and associated tuber tissue following 24 h of treatment of 'Russet Burbank' tubers with (*E*)-2-nonenal, nonanal, (*E*)-2-nonen-1-ol, and 1-nonanol at four concentrations as specified in the *x*-axis labels. Sprouts were ca. 2 cm long at the time of treatment. Data are means of 8 sprout/tissue disks randomly chosen from 28 to represent four tubers. Vertical bars are SE.

conversion of the parent compound, as the concentration of 3-nonen-2-one recovered from sprouts and surrounding tissue increased with application rate from 0.25 to 0.75 mmol kg^{-1} tuber fresh weight. 2-Nonanone applied to tubers was reduced to 2-nonanol, and the efficiency of conversion (mol % recovered) decreased with increasing concentration ($P < 0.001$). However, when 2-nonanol was applied, only a small amount was oxidized to 2-nonanone.

When (*E*)-2-nonenal was applied at four rates, the most complete catabolism occurred at the two lowest rates (0.125 and 0.25 mmol kg^{-1} fresh weight), and, regardless of the rate applied, (*E*)-2-nonen-1-ol was the dominant species recovered 24 h after treatment (Figure 3). Nonanal was always the least abundant product, and when applied to sprouted tubers, it was predominately converted to the primary alcohol, 1-nonanol, in a concentration-dependent manner ($P < 0.001$). Neither 1-nonanol nor (*E*)-2-nonen-1-ol was substantially metabolized at any application rate.

Tuber Residue Studies. Residues resulting from the application of 3-nonen-2-one and (*E*)-2-nonenal were measured in whole tubers over a 28 day period (Figure 4). 3-Nonen-2-one, 2-nonanone, and 2-nonanol were not detected in nontreated 'Ranger Russet' tubers. The concentration of 3-nonen-2-one was 41 nmol g^{-1} fresh weight in tuber tissue immediately following treatment (zero time), but declined to 9 nmol g^{-1} fresh weight within 24 h of storage at 9 °C (95% RH) (Figure 4A). By 28 days after treatment, 3-nonen-2-one had dropped to 0.4 nmol g^{-1} fresh weight. The post-treatment concentration of 2-nonanone also reached a maximum (4 nmol g^{-1} fresh weight) directly following treatment and then fell rapidly, to 0.6 nmol g^{-1} fresh weight by 28 days. The trend for 2-nonanol residue was unique in that the maximum concentration associated with initial treatment was not reached until 24 h after the tubers had been removed from the treatment barrels (i.e., the initial treatment with 3-nonen-2-one had ended). 2-Nonanol residue then declined slightly before recovering to an average of approximately 23 nmol g^{-1} tuber fresh weight for the duration of study.

The overall levels of (*E*)-2-nonenal and associated metabolite residues were substantially lower than those of 3-nonen-2-one, but similar trends were observed over the 28 day post-treatment interval (Figure 4B). Trace quantities of (*E*)-2-nonenal and nonanal were detected in some samples of nontreated tissue (1 day prior to treatment). These background levels averaged approximately 0.1 nmol g^{-1} fresh weight for both compounds and were ignored for the purposes of quantitation of the residual species arising from treatment with (*E*)-2-nonenal. The data in Figure 4B therefore include this background. The (*E*)-2-nonenal concentration was highest immediately following removal of tubers from the treatment barrel (9.5 nmol g^{-1} fresh weight at zero time) and rapidly declined to the background level (ca. 0.1 nmol g^{-1} fresh weight) by day 7. Nonanal, 1-nonanol, and (*E*)-2-nonen-2-ol reached maximum levels 24 h after removal from the treatment barrel. Nonanal and 1-nonanol declined slightly after this point, ultimately reaching 0.6 and 0.1 nmol g^{-1} fresh weight, respectively, on day 28. (*E*)-2-Nonen-1-ol was the most concentrated and persistent breakdown product of (*E*)-2-nonenal, reaching a maximum concentration of 2.4 nmol g^{-1} fresh weight on day 1 and then gradually declining to 1.2 nmol g^{-1} fresh weight over the 28 day post-treatment storage period at 9 °C.

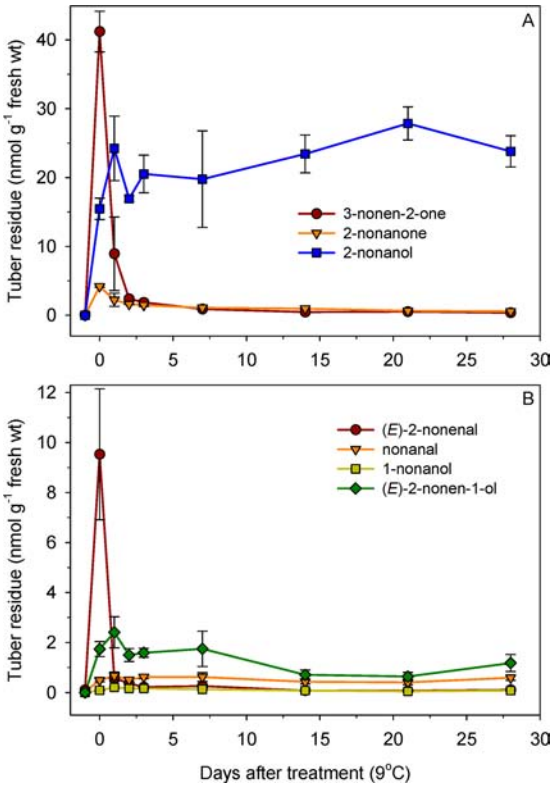

Figure 4. Time-dependent changes in tuber residue levels resulting from treatment of 'Ranger Russet' tubers for 24 h with 3-nonen-2-one (A) or (*E*)-2-nonenal (B) at 0.75 mmol kg^{-1} tuber fresh weight. Tubers were removed from treatment barrels at zero time and stored at 9 °C (95% RH) over the 28 day interval. Each data point is the average of 12 tubers ± SE.

Long-Term Efficacy. Application dates of (*E*)-2-nonenal and 3-nonen-2-one to terminate sprout growth in 'Russet Burbank', 'Umatilla Russet', and 'Ranger Russet' tubers were based on sprouting characteristics of the individual cultivars. Inhibitors were applied at the peeping stage (sprouts ≤2 mm). 'Ranger Russet' tubers have the shortest dormancy of the three cultivars and required initial treatment in January (104 DAH), whereas 'Umatilla Russet' and 'Russet Burbank' tubers did not begin sprout growth until mid and late February, respectively. First application for these cultivars was 135 DAH. The sprout-inhibiting efficacy of this first treatment was similar for both (*E*)-2-nonenal and 3-nonen-2-one, and therefore the second application of both chemicals was made on the same day to a given cultivar (173 DAH for 'Ranger Russet' and 202 DAH for 'Umatilla Russet' and 'Russet Burbank'). This second application occurred when new sprouts began to emerge (peeping stage) from the previously treated tubers.

Duration of the sprout-free period following initial applications of either 3-nonen-2-one or (*E*)-2-nonenal was about equal for all three cultivars, averaging 68 days in 2004 (Figure 5). This efficacy compares well with results for 'Ranger Russet' and 'Umatilla Russet' (more aggressive sprouting cultivars) in trials from other years; however, the duration of inhibition in 'Russet Burbank' following a single application can be as much as 3.5 months (data not shown). Following a second application of either chemical, 'Russet Burbank' and 'Umatilla Russet' tubers remained sprout-free for a further 115 days. Beyond initial dormancy break at 135 DAH, the total sprout free interval for these two cultivars with two applications of either compound was an additional 182 days. *E*-2-Nonenal was slightly more effective than 3-nonen-2-one in suppressing sprout growth in 'Ranger Russet', extending the sprout-free period by 213 versus 187 days, respectively, following initial dormancy break (104 DAH) with two applications.

DISCUSSION

Using a simple bioassay involving nondormant potato tubers, a number of volatile carbonyl compounds were effectively screened for toxicity to very small, etiolated sprouts. Storage of the nondormant tubers at 16 °C (95% RH) in darkness following chemical application challenged the potential efficacy of these test compounds as sprout inhibitors, as sprout growth is rapid and vigorous at this temperature. All 8-, 9-, and 10-carbon α,β-unsaturated ketones and aldehydes screened in this study effectively killed the sprouts present at the time of treatment and, depending on the concentration applied, inhibited regrowth. Data from the bioassay helped identify sublethal dosages appropriate for subsequent studies of metabolism by sprout and associated tuber tissues.

In longer term studies designed to reflect commercial storage durations, single applications of 3-nonen-2-one and (*E*)-2-nonenal were both effective in killing actively growing sprout tissue and suppressing further growth for at least 2 months in several potato cultivars. However, a second treatment was required for extended full-season sprout control. It was apparent from these studies that the α,β-unsaturated carbonyls act quickly to necrotize developing etiolated sprout tissue but at the concentrations tested had no permanent inhibitory effect on inactive meristems. Therefore, regrowth is to be expected, and this regrowth is partly due to the phenomenon of apical dominance.

Tubers emerging from dormancy typically exhibit at least some degree of apical dominance, the magnitude of which depends on the cultivar and physiological age and condition of the tuber. Apical dominance is the correlative inhibition of sprout growth in the lateral eyes (nodes) by sprouts that first emerge from dormancy and begin developing at the apical end of the tuber. The initial application of 3-nonen-2-one and (*E*)-2-nonenal primarily terminated only those sprouts that had begun to elongate, but had much less of an effect on the lateral meristems that were still inactive and had not yet begun growth. Sprout fresh weight increase following the first chemical treatment was therefore primarily due to the growth of sprouts that had been released from apical dominance. By 8−12 weeks following the initial application, more meristems became active, producing peeping sprouts that were highly susceptible to the toxic (fully necrotizing) effects of the α,β-unsaturated carbonyls. These sprouts could thus now be successfully terminated, resulting in an even longer period of sprout suppression following the second application. Whereas the need for reapplication is inconvenient for season-long storage of fresh market and processing potatoes, the temporary inhibition resulting from one application of α,β-unsaturated carbonyls could be advantageous in the seed potato industry to terminate premature sprouting in storage while still allowing growth at the time of field planting.

α,β-Unsaturated ketones (8−10-carbon) followed a common metabolic fate in potato sprout tissue as did the three aldehydes. Whole tubers also demonstrated the ability to metabolize the 9-carbon aldehyde and ketone species efficiently. The analytical methodology employed in this study (SPME) permits only collection and identification of

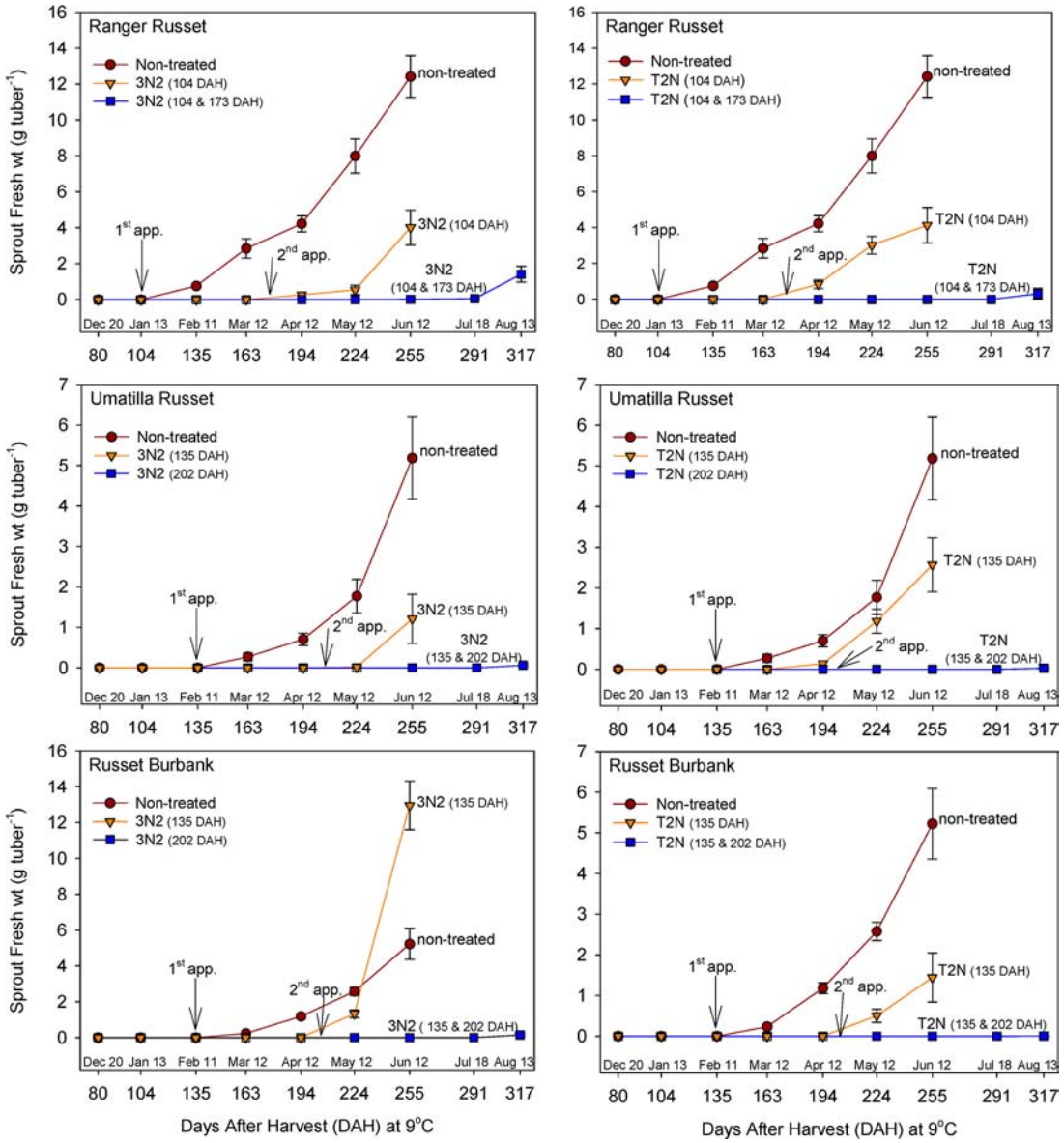

Figure 5. Effects of single and dual applications of 3-nonen-2-one (3N2) (left) or (*E*)-2-nonenal (T2N) (right) on sprout growth of 'Ranger Russet' (top), 'Umatilla Russet' (middle), and 'Russet Burbank' (bottom) potatoes. 3-Nonen-2-one and (*E*)-2-nonenal were applied at 0.75 mmol kg^{-1} of tubers. The tubers were grown at Othello, WA, wound-healed for 10 days at 12 °C following harvest, and stored at 9 °C (95% RH) for the durations indicated. Timings of the first and second applications (app) of sprout inhibitors are indicated (arrows and in legend). Each data point is the average of 12 tubers ± SE.

volatile metabolites; therefore, fully oxidized products of metabolism (e.g., R−COOH from the aldehydes), if any, would not be detected. The metabolism of all six species included the reduction of the carbonyl oxygen to a hydroxyl group and the addition of H across the α,β double bond. Although this double bond was preserved in the case of the (*E*)-2-enols recovered from the unsaturated aldehyde treatments, we did not find the unsaturated secondary alcohol in tissue exposed to the unsaturated ketones. It is possible this compound was either a minor component of the metabolic pool with a concentration below the sensitivity of our analyses or rapidly converted to the secondary alcohol.

Transformations such as those reported above have been documented in other plant species and may represent a mechanism of detoxification. Mano et al.[9] demonstrated the relatively high toxicity of α,β-unsaturated aldehydes to photosynthetic carbon fixation compared to their corresponding alcohols and saturated aldehydes. Schultz et al.[10] used an aquatic species (*Tetrahymena pyraformis*) population growth bioassay to compare the toxicity of a large number of saturated and unsaturated ketones. Alkenones and alkynones were more toxic overall than the saturated species, which they interpreted as increased bioreactivity. It is possible that higher plants such as potato transform α,β-unsaturated ketones by similar mechanisms to render them less reactive.

Several examples exist for the reduction of double bonds in unsaturated aldehydes and ketones. Hamilton-Kemp et al.[11] applied 3-nonen-2-one vapors to strawberry fruit in a manner similar to that described in our studies for potato tubers and identified 2-nonanone as the sole metabolite in the headspace of the treatment chamber. Also in their study (*E*)-4-hexen-3-one was metabolized to 3-hexanone, confirming the ability of

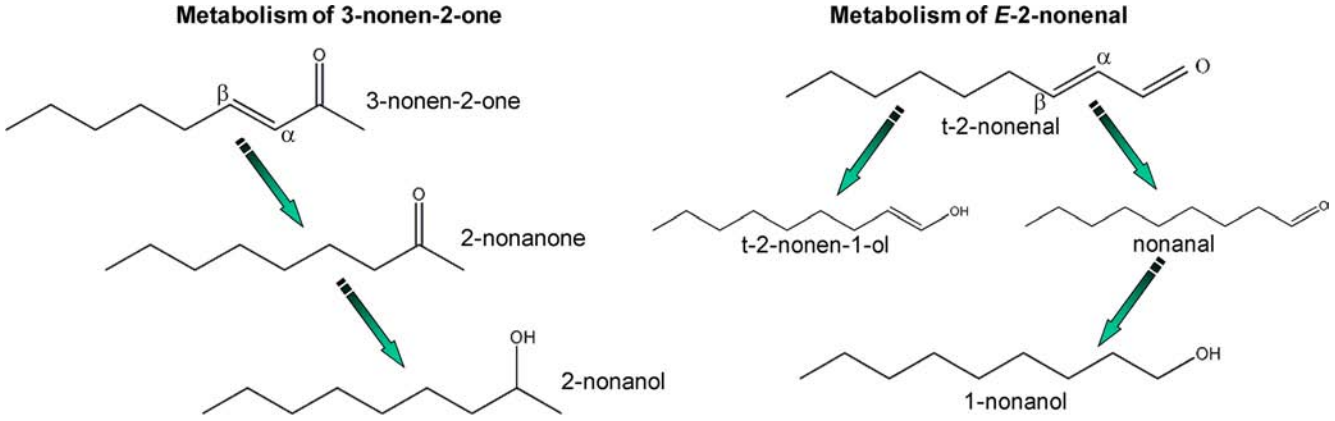

Figure 6. Schematic illustrating the pathways of metabolism of 3-nonen-2-one (left) and (*E*)-2-nonenal (right) by potato sprout and tuber tissues. Six 8−10-carbon aliphatic aldehydes and ketones were each metabolized to the corresponding alcohols and saturated carbonyls as shown for 3-nonen-2-one and (*E*)-2-nonenal (see Figures 2−4).

the fruit to reduce double bonds in the *α,β* position of aliphatic ketones. A recombinant alkenal reductase derived from *Arabidopsis* (At-AER) reduced the *α,β* double bond of 3-nonen-2-one to produce 2-nonanone without further reduction to the alcohol.[12] 3-Buten-2-one and 3-penten-2-one also served as substrates.[13] Hambraeus and Nyberg cloned an alkenal hydrogenase (ALH1) from barley for expression in *Escherichia coli*.[14] 1-Octen-3-one functioned as an electron acceptor when incubated with the purified ALH1 and NADPH, implying reduction of the *α,β* double bond. The equivalent reaction converting (*E*)-2-aldehydes to their saturated analogues was also demonstrated in the latter studies. The barley alkenal hydrogenase converted (*E*)-2-nonenal to nonanal.[14] Mano et al. demonstrated hydrogenation of the double bond of 4-hydroxy-(*E*)-2-nonenal (HNE) in leaf extracts of *Arabidopsis*.[13] They also showed that the recombinant form of the alkenal reductase (AER) could catalyze reduction of the *α,β* double bond of (*E*)-2-alkenals in vitro with a high degree of specificity for 9-carbon species. Expression of At-AER in tobacco conferred a higher activity of NADPH-dependent HNE reduction in leaf extracts.[12] In our study, unsaturated alcohols were not a preferred substrate for the enzyme reducing double bonds of unsaturated carbonyls in potato sprouts (Figure 3), as very little conversion to nonanol was measured when sprouts were exposed to (*E*)-2-nonen-1-ol.

The reduction of carbonyls in both the enones and enals was observed for all compounds applied to potato sprouts. In the case of *α,β*-unsaturated ketones only the saturated secondary alcohol was detected in sprouts treated with 3-octen-2-one, 3-nonen-2-one, or 3-decen-2-one. Examination of the 9-carbon species demonstrated the conversion of 2-nonanone to 2-nonanol (Figure 2) with the greatest production of the alcohol at the lowest dose. *α,β*-Unsaturated aldehydes were converted to the analogous primary alcohol, but unlike the ketones, the unsaturated primary alcohol was also detected (Table 1). Precursor−product studies with the 9-carbon species detailed in Figure 3 indicate that the saturated primary alcohol was derived principally from the saturated aldehyde. In strawberries, (*E*)-2-hexenal was metabolized to (*E*)-2-hexen-1-ol and 1-hexanol,[11] which is consistent with our results for 8−10-carbon enals applied to potato sprouts.

The aldo-keto reductase superfamily contains enzymes capable of catalyzing the reductions of aldehydes to alcohols.[15] These enzymes are more thoroughly characterized in humans than in plants; however, two enzymes from *Arabidopsis* demonstrated the ability to reduce (*E*)-2-hexenal.[16] Intact tissues of carrot and *Manihot* sp. are capable of reducing aliphatic ketone substrates to the corresponding secodary alcohols.[17,18] Saturated and unsaturated aldehydes were effective substrates for an aliphatic aldehyde reductase in coriander.[19] The KCR3 gene from cotton was cloned into *E. coli*, and the recombinant enzyme recovered reduced C3 to C13 saturated aldehydes.[20] Montesano et al.[21] described the enzymatic activity of the DRD-1 gene obtained from a potato leaf cDNA library. The recombinant enzyme converted hexanal, octanal, (*E*)-2-hexenal, (*E*)-2-nonenal, and (*E*)-2-(*Z*)-6-nonadienal to their corresponding alcohols. In that study, the reverse reaction of nonanol to nonanal was significantly slower and confirms what we found in tuber and sprout tissue where <3% of applied 1-nonanol was converted to nonanal (Figure 3).

The application of 3-nonen-2-one and its metabolites individually to potato sprouts followed by subsequent analysis of the products indicated that the pathway of breakdown for *α,β*-unsaturated ketones in potato tubers proceeds as *α,β*-unsaturated ketone to saturated ketone to saturated secondary alcohol (Figure 6). On the basis of analogous data, we postulate two possible metabolic fates for (*E*)-2-nonenal: *α,β*-unsaturated aldehyde to saturated aldehyde to saturated primary alcohol or *α,β*-unsaturated aldehyde to unsaturated primary alcohol (Figure 6). Interestingly, all of these metabolites have efficacy as sprout inhibitors; however, their toxicity to sprouts is much lower than that of the *α,β*-unsaturated carbonyl parent compounds (data not shown).

The high consumption rate of potatoes in North America means low regulatory tolerance for pesticide residues. To address the issue of residues, we followed changes in the levels of 3-nonen-2-one, (*E*)-2-nonenal, and their respective metabolites over a 28 day period in the tissue of treated tubers. The metabolism of the parent compounds progressed as predicted on the basis of the results of the metabolite study. The persistence of 2-nonanol following treatment of tubers with 3-nonen-2-one (Figure 4A) was expected given that sprout tissue was unable to convert this compound to a significant degree. In (*E*)-2-nonenal-treated tubers, (*E*)-2-nonen-1-ol was the predominant residue 7 days following application (Figure 4B). Although 1-nonanol was identified as an alternative end product in the metabolic pathway study, it is derived from nonanal, which is not produced in significant quantities. The

lower reactivity of these products may translate into higher tolerance for residues by regulatory agencies; however, approval for registration will hinge upon thorough human toxicity and environmental impact assessments.

In summary, we have demonstrated the ability of certain α,β-unsaturated carbonyl compounds to terminate growth of etiolated sprouts developing from dark-stored potato tubers. The feasibility of using the 8−10-carbon species commercially for sprout suppression may be bolstered by the rapid metabolism of excess material to relatively less reactive aliphatic alcohols. With proper timing of application, long-term inhibition of sprout growth in a commercial storage environment is likely possible with multiple applications of these compounds.

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Notes

The authors declare the following competing financial interest(s): The sprout inhibiting technology reported in this manuscript has been patented by the authors and WSU. WSU has licensed the rights to the technology to AMVAC Chem Co. and they are currently developing this chemistry for commercial use.

ABBREVIATIONS USED

DAH, days after harvest; 3N2, 3-nonen-2-one; T2N, (*E*)-2-nonenal; PDMS/DVB, polydimethylsiloxane/divinylbenzene; ALH1, alkenal hydrogenase 1; HNE, 4-hydroxy-(*E*)-2-nonenal; AER, alkenal reductase; 3D2, 3-decen-2-one; GC-FID, gas chromatography−flame ionization detection.

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