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The Involvement of Gibberellins in 1,8-Cineole-Mediated Inhibition of Sprout Growth in Russet Burbank Tubers

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Abstract The involvement of gibberellins in 1,8-cineole-mediated inhibition of tuber sprout growth was investigated in non-dormant field- and greenhouse-grown tubers of Russet Burbank. Continuous exposure of tubers to cineole in the vapor-phase resulted in a dose-dependent inhibition of sprout growth. Comparative studies using plant bioassay systems whose growth was differentially dependent on cell division, cell elongation, or both demonstrated that cineole had no direct effect on either process. Of the assays used, only cineole-mediated inhibition of etiolated hypocotyl growth mirrored the inhibition of tuber sprout growth which suggested an effect on gibberellin synthesis or action. Both GA_{19} and GA_{20} were detected in extracts prepared from control sprouts but only GA_{19} was found in extracts prepared from cineole treated sprouts. Exogenous GA_3, GA_{20}, and GA_1 (but not GA_{19}) reversed cineole-mediated sprout growth inhibition. Expression of genes encoding key GA metabolic enzymes was altered by cineole treatment in a manner consistent with diminished endogenous GA content. Collectively, these results suggest that the inhibition of sprout growth by low vapor-phase concentrations of cineole is in part a result of impaired GA biosynthesis resulting in a reduction in bioactive GA content.

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Resumen Se investigó el involucramiento de giberelinas en la inhibición del crecimiento del brote de tubérculo mediado por 1,8-cineole, en tubérculos en dormancia del campo y del invernadero de Russet Burbank. La exposición continua de los tubérculos a cineole en la fase de vapor dio por resultado una inhibición del crecimiento del brote dependiente de la dosis. Estudios comparativos usando sistemas de bioensayos de plantas cuyo crecimiento era diferencialmente dependiente de la división celular, elongación celular, o ambas, demostraron que cineole no tuvo efecto directo en ninguno de los procesos. De los ensayos usados, solamente la inhibición mediada por cineole de crecimiento de hipocotilo elongado, reflejó la inhibición del crecimiento del brote de tubérculo, lo cual sugirió un efecto en la síntesis o acción de giberelina. Se detectaron GA19 y GA20 en extractos preparados de los brotes testigo, pero solo el GA19 se encontró en los extractos de brotes tratados con cineole. La aplicación exógena de GA3, GA20 y GA1 (no GA 19) revirtió la inhibición del crecimiento del brote mediada por cineole. Se alteró la expresión de los genes que codifican las enzimas metabólicas clave de GA mediante el tratamiento con cineole de manera consistente con la disminución del contenido endógeno de GA. Colectivamente, estos resultados sugieren que la inhibición del crecimiento del brote por bajas concentraciones de cineole en fase de vapor es en parte un resultado de la biosíntesis debilitada de GA, resultando en una reducción del contenido de GA bioactivo.

Keywords Mono-terpene · Potato · *Solanum tuberosum* · Sprouting

Abbreviations

(DMSO)	Dimethyl sulfoxide
(GA)	Gibberellin

(MS)	Murashige Skoog
(PCR)	Polymerase Chain Reaction
(UPLC ESI-MS/MS)	ultra-high-performance liquid chromatography-electrospray ionization tandem mass spectrometry

Introduction

At harvest and for an indeterminate period thereafter, potatoes are in a state of physiological dormancy and will not sprout (Burton 1989). During storage, physiological dormancy is lost in a temperature-dependent manner. With the exception of seed potatoes (stored below 4 °C), the loss of dormancy in potatoes destined for processing and stored at temperatures in excess of 9 °C is followed by the onset of sprout growth. Sprout growth is accompanied by wholesale changes in tuber physiology leading to a loss of nutritional and process qualities. As a result, suppression of sprout growth is an essential component of successful potato storage management (Kleinkopf, et al. 2003).

In potatoes intended for the process market, tuber sprouting is controlled primarily through the use of sprout suppressing chemicals. Both synthetically derived and natural-product-based sprout suppressants are registered for use in commercial storages (Fig. 1). The most widely used compound, chlorpropham (CIPC), is a repurposed herbicide that inhibits sprout growth by blocking cell division (Moreland 1980). The sprout suppressant maleic hydrazide (applied as a pre-harvest foliar spray) is translocated to the tubers where it inhibits postharvest sprouting by interfering with nucleic acid biosynthesis (Yu, et al. 1978). More recently, three natural product based compounds (clove oil, carvone, trans-3-decen-2-one) have been registered as sprout control agents. All three compounds inhibit sprout growth by physically damaging (burning) sprout meristems (Baydar and Karadogan 2004; Teper-Bamnolker, et al. 2010). Another compound, 1,4-dimethylnaphthalene, naturally present in tubers (Burton and Meigh 1971), reversibly inhibits sprout growth in part by disrupting cell cycle progression and ultimately inhibiting cell division (Campbell, et al. 2012).

In addition to these registered materials, a number of low-molecular weight volatile oils have been reported to exert sprout suppressing activities (Kleinkopf, et al. 2003). In a follow-up of pioneering studies conducted by Meigh (1969; Meigh et al. 1973), the sprout inhibiting properties of a selected series of plant-derived monoterpenes were examined (Vaughn and Spencer 1991). At saturating vapor concentrations, 1,8-cineole (1,3,3-Trimethyl-2-oxabicyclo [2,2,2] octane, Fig. 1; henceforth cineole) completely suppressed sprouting and resulted in visible phytotoxicity. A constituent of a number of plant essential oils, cineole is a known allelopathic agent that effectively inhibits both seed germination and seedling growth (Romagni et al. 2000). In addition, plant monoterpenes are potent anti-microbials and have been reported to inhibit the in vitro proliferation of several human cancer cell lines (Crowell, et al. 1994). However, their mode of action in these systems has not been defined.

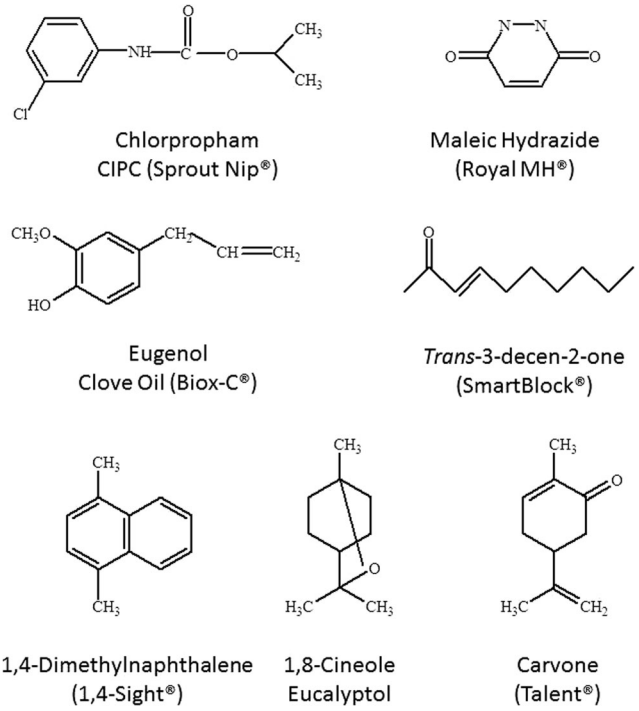

Fig. 1 Chemical structures of cineole and other registered sprout inhibitors

During the course of preliminary experiments using very low vapor-phase concentrations of cineole, inhibition of tuber sprout growth was found to be completely reversible (data not shown). This observation suggested a novel non-herbicidal mechanism of action quite unlike that exhibited by other registered sprout inhibitors. In this paper, this hypothesis is explored further and the results obtained suggest that cineole inhibits sprout growth at least in part through reversible inhibition of gibberellin (GA) biosynthesis.

Materials and Methods

Plant Material, Chemicals, and Experimental Procedures

Two types of potato tubers (*Solanum tuberosum* L. cv. Russet Burbank) were used in these studies. Field-grown seed tubers were obtained from a commercial grower within 2 days of harvest. Tubers were cured in the dark at room temperature for 2 weeks and were placed into storage (4 °C). Fully cured, greenhouse-grown pre-nuclear seed minitubers (5–10 g/tuber) were obtained from a commercial grower (Valley Tissue Culture, Halstad, MN, USA) and were immediately placed in cold (4 °C) storage. In both cases, tubers were incubated in the dark

(20 °C) for 3 days prior to use. All tubers used in these studies had been stored for a minimum of 7 months and were completely non-dormant (i.e., > 5 mm sprout growth after 14 days at 20 °C). GA_3 and 1,8-cineole were purchased from Sigma (St. Louis, MO, USA) and GA_{19}, GA_{20}, and GA_1 were purchased from OlChemIm (Olomouc, Czech Republic). All experiments described in this paper were conducted a minimum of two times with comparable results. Unless otherwise noted, all treatments within an experiment were performed with ≥3 biological replicates. In all cases, data from a typical experiment are presented as means ± SE. Statistical analysis was conducted using SAS 9.0 to determine least significant differences (LSD) at $P = 0.05$.

Cineole-Mediated Growth Inhibition Studies

After equilibration, minitubers were hand washed and dried in the dark at room temperature. Ten washed minitubers were placed in 4.5 L acrylic chambers containing a beaker with 50 mL deionized water to maintain humidity. Liquid cineole was pipetted onto cotton wadding in a second beaker which was immediately placed into the chamber and the chamber was sealed with electrician's tape. All cineole treatment concentrations reported indicate the volume of liquid cineole added per liter of chamber volume prior to sealing. The sealed chamber was incubated in the dark (20 °C). After 7 days, the chambers were opened and vented for 30 min, fresh cineole was added to new cotton wadding, and the chambers were resealed. After 14 days of treatment, the length of the longest sprout per minituber was measured to the nearest mm.

A similar method was used for the comparative inhibition studies. The assay systems selected for these comparisons and the underlying cellular basis (es) for growth were: seed germination (elongation), etiolated hypocotyl growth (elongation), etiolated primary root growth (division and elongation), and in vitro callus growth (division). For these studies, all tissues treated with the same cineole concentration were placed in the same acrylic chamber thereby eliminating any experimental differences in treatment concentration. Minitubers were prepared as described above. Four equally sized blocks of potato callus (maintained on MS media containing 0.4 mg L^{-1} α-naphthalene acetic acid and 5 mg L^{-1} 2,4-dichlorophenoxyacetic acid) were transferred to fresh media in covered 9 cm petri dishes 7 days prior to use. True potato seed was placed on filter paper wetted with 10ug/L GA_3 (sufficient to break seed dormancy but not affect subsequent seedling growth; Franklin and Wareing 1960) in covered 9 cm petri dishes (20 seeds/ dish). Percent germination was determined after 7 days. The length of the longest sprout, final hypocotyl and root lengths (to the nearest mm), and fresh weight of individual callus pieces were determined after 14 days.

GA Reversal Studies

After washing, a small cavity (5 mm deep) was made immediately below the apical bud in each minituber using a 16G needle. Five μL of DMSO alone or containing 10 μg GA_3, GA_{19}, GA_{20}, or GA_1 was introduced into the cavity using a syringe. Groups of ten control and GA-treated minitubers were then placed in 10 L acrylic chambers containing a beaker with 50 mL deionized water. Cineole (at the indicated concentration) was pipetted onto cotton wadding in a second beaker and immediately placed into the chamber which was then sealed. After 14 days of dark incubation (20 °C), the length of the longest sprout was determined to the nearest mm.

Determination of GA Content

After equilibration and washing, groups of twenty field-grown tubers were placed in 10 L acrylic chambers containing a beaker with 50 ml deionized water. A second beaker containing ca. 100 g activated charcoal (control) or cineole (30 μL) was placed into each chamber immediately before sealing. After 14 days of dark incubation (20 °C), the tubers were removed from the chambers and, after measuring sprout length of 10 representative tubers, all sprouts >5 mm long (ca. 0.6–1.3 g FW) were harvested, frozen in liquid nitrogen, and freeze-dried. After drying, the sprouts were pulverized using a mechanical mill and 50 mg aliquots were prepared for hormone analysis. Hormone analysis was performed on triplicate biological replicates by UPLC ESI-MS/MS at the Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, SK (Chiwocha et al. 2003).

Effects of Cineole on GA-related gene expression

Groups of 50 washed and air-dried minitubers were placed in 10 L acrylic chambers containing a beaker with 50 mL deionized water. A second beaker containing ca. 100 g activated charcoal (control) or cineole (15 μL) was placed into each chamber immediately before sealing. Chambers were incubated for 14 days in the dark (20 °C). Sprouts were harvested, frozen in liquid nitrogen, and stored at −80 °C until used. RNA isolation, cDNA preparation, and qRT-PCR analysis were performed exactly as described previously (Suttle et al. 2014) using gene-specific primer pairs (Supplemental Table 1). The Relative transcript abundances were calculated by the $\Delta\Delta C_T$ method (Tsai et al. 2006) using the mean of C_T values for the two housekeeping genes (EF1α and actin) as normalizing factors.

Results

Vapor-phase exposure of non-dormant tubers to low concentrations of cineole resulted in a dose-dependent inhibition of sprout growth (Fig. 2). Fifty percent inhibition of sprout growth was observed at a cineole dose of ca. 1.6 μL L^{-1} and visible growth was almost completely (>87 %) suppressed at a cineole concentration of 6.2 μL L^{-1}. After 2 weeks of exposure to these low concentrations of cineole, phytotoxicity (tip necrosis) was not observed at any dose tested and sprout growth resumed following termination of exposure (data not shown).

At the cellular level, sprout growth is a result of both cell division and cell elongation. In order to determine the cellular basis (es) for cineole-induced inhibition of sprout growth, the effects of cineole in assay systems that differ in the cellular bases of growth were examined next. Four assays were selected for comparison: potato seed germination (elongation), etiolated potato seedling hypocotyl extension (elongation), etiolated potato seedling root growth (division and elongation), and potato cell callus growth (division). In conducting these comparisons, all biological assay systems were enclosed in the same treatment chamber thereby assuring equivalent dosing. At concentrations of 6.2 μL L^{-1} or less, cineole had no effect on true potato seed germination (data not shown). Hypocotyl elongation was inhibited by cineole in a dose-dependent manner with exposure to 6.2 μL L^{-1} cineole resulting in greater than 75 % inhibition (Fig. 3). In contrast, concentrations of cineole ≤6.2 μL L^{-1} had no demonstrable effects on potato seedling root growth or potato callus growth.

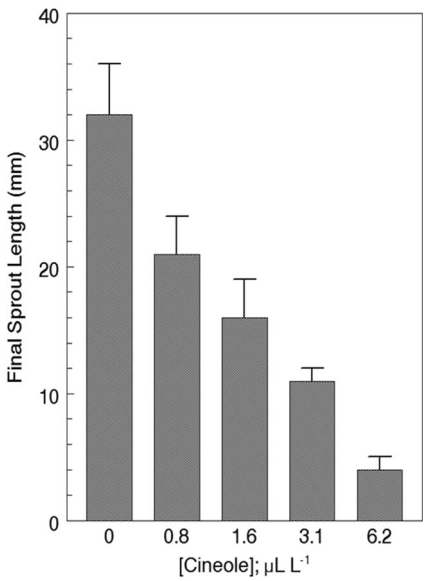

Fig. 2 Effects of increasing vapor-phase concentrations of cineole on tuber sprout growth. After 14 days of continuous exposure, the length of the longest sprout on each minituber was measured to the nearest mm. Data presented are means ± SE ($n = 10$)

The failure to observe a consistent pattern of inhibition of either cell division or elongation suggested a more subtle mode of action for cineole. Hypocotyl elongation is a gibberellin (GA)-dependent process and is the basis for a well-known and highly specific bioassay for GA (Franklin and Wareing 1960). The observed inhibition of etiolated hypocotyl growth (which mirrored that of sprout growth) by cineole without a corresponding effect on the other assays used is consistent with an effect on GA biosynthesis and/or action. This possibility was explored by determining the ability of exogenous GA to reverse cineole-mediated sprout growth inhibition and by determining the effects of cineole on the endogenous contents GA.

First, the effects of GA_3, a highly bioactive (but non-native) GA on sprout growth in control and cineole-treated tubers was determined. Application of 10 μg GA_3 to control or untreated tubers had a small but non-

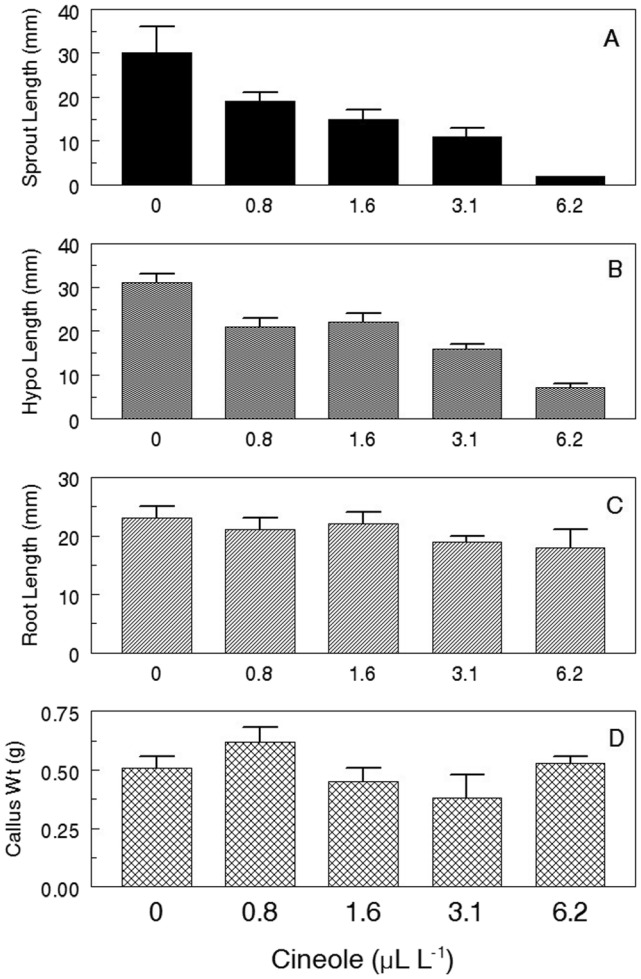

Fig. 3 Comparative effects of increasing vapor-phase concentrations of cineole on final tuber sprout length (panel **a**), etiolated potato seedling hypocotyl length (panel **b**), etiolated potato seedling primary root length (panel **c**), and potato callus fresh weight (panel **d**). All measurements were made after 14 days of continuous treatment. Data presented are means ± SE ($n = 10$, sprout, hypocotyl, root lengths or 4, callus fresh weight)

significant effect on the length of the longest sprout (Fig. 4). As before, cineole treatment resulted in a dose-dependent decrease in sprout growth. Application of GA_3 to tubers treated with ≤1.5 μL L^{-1} cineole completely abolished the inhibition of sprout growth. At the highest concentration of cineole (3 μL L^{-1}), application of GA_3 significantly increased sprout growth but was not able to completely reverse cineole-mediated inhibition.

Next, the effects of cineole on the endogenous contents of selected GAs were determined in tuber sprouts following 2 weeks of treatment. In potato tissues (including tuber sprouts), the endogenous GAs are members of the early-13-hydroxylation pathway (Fig. 5) leading from GA_{19}, through GA_{20} to GA_1, which is considered the biologically active hormone, and ultimately to GA_8 which is biologically inactive (Jones et al. 1988; Suttle 2004). The endogenous contents of these GA were determined by mass spectrometry. Both GA_{19} and GA_{20} were detected in untreated sprouts but the levels of GA_1 and GA_8 were below the limits of detection (Table 1). Only GA_{19} was detected in cineole-treated sprouts and the endogenous levels of the remaining GAs were all below the limits of detection. These results suggest that cineole treatment blocked the conversion of GA_{19} to GA_{20} which would result in a corresponding decline in the endogenous content of bioactive GA_1 and reduction of GA-dependent sprout growth. The failure to observe an increase in GA_{19} content in cineole-treated tubers was unexpected and may have been a result of feedback inhibition in the GA biosynthesis pathway in these tissues.

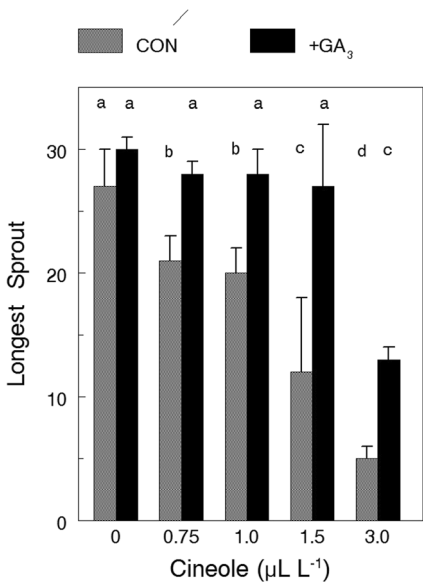

Fig. 4 Effects of GA_3 on cineole-mediated inhibition of sprout growth. Minitubers were treated with 5 μL DMSO ± 10 μg GA_3. Minitubers were continuously exposed to increasing concentrations of cineole for 14 days. The length of the longest sprout per minituber was measured to the nearest mm. Data presented are means ± SE ($n = 10$). Means with the same letter are not significantly different at $P = 0.05$

This possibility was examined by determining the abilities of the terminal three members of the early-13-hydroxylation pathway including GA_{19} and GA_{20} (the two immediate biosynthetic precursors of GA_1) as well as GA_1 to reverse cineole-induced sprout growth inhibition. In untreated (control) tubers, only GA_1 treatment resulted in a significant (ca. 45 %) increase in sprout growth; GA_{19} and GA_{20} were without effect (Fig. 6). In tubers treated with 1 μL L^{-1} cineole, both GA_{20} and GA_1 completely abolished cineole-mediated sprout growth inhibition restoring sprout growth to control levels. Treatment with GA_{19} had no effect. A similar (albeit less pronounced) effect of exogenous GA was observed in tubers treated with 1.5 μL L^{-1} cineole. Both GA_{20} and GA_1 significantly increased sprout growth in cineole treated tubers but not to the extent seen in tuber treated with the lower dose. In these tubers, GA_{19} treatment elicited a small but insignificant increase in sprout growth. These results are consistent with the hypothesis that, at low doses, cineole-mediated suppression of sprout growth occurs primarily as a result of a

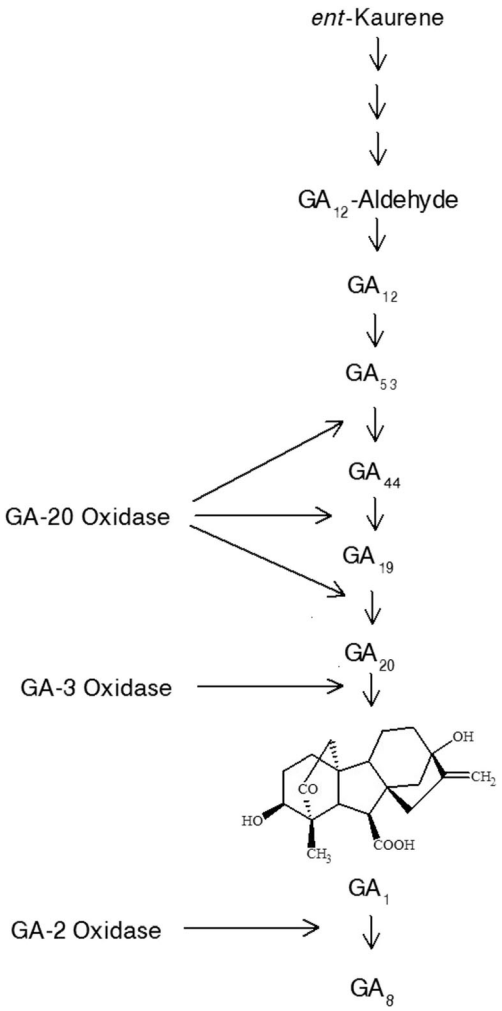

Fig. 5 Biosynthetic pathway of GA_1 biosynthesis. Steps catalyzed by GA-20 oxidase, GA-3 oxidase, and GA-2 oxidase are indicated

Table 1 The effects of cineole treatment on the endogenous contents of GA_{19}, GA_{20}, GA_1, and GA_8 in potato tuber sprouts

GA Content (ng g^{-1} DW)				
Treatment	GA_{19}	GA_{20}	GA_1	GA_8
Control	67 ± 3[1]	19 ± 3	nd[2]	nd
Cineole	58 ± 7	nd	nd	nd

[1] Mean ± SE ($n = 3$)

[2] Not detected

reduction in bioactive GA_1 content that in turn results from an inhibition of the conversion of GA_{19} to GA_{20}.

The regulation of endogenous GA content is under strict developmental and environmental control often operating at the level of transcription (Sponsel and Heddon 2004). Although many potential sites of regulation exist, in most instances homeostatic control of GA content is achieved by modulated expression of genes encoding GA-20 oxidase, GA-3-oxidase and/or GA-2-oxidase (Fig. 5; Yamaguchi 2008). In most plants, these proteins are encoded by small gene families (Yamaguchi 2008). The effect of cineole treatment on the expression of these genes in elongating sprouts was examined next by PCR using gene-specific primers. Cineole treatment had no significant effects on the expression of *StGA20ox1* or *StGA20ox2* but elicited a small increase in *StGA20ox3* expression. Expression of *StGA3ox1* was unaffected by cineole treatment while expression of *StGA30x2* was decreased. Expression of genes encoding the catabolic enzyme StGA2ox was also differentially affected by cineole treatment. Cineole had no effect on the expression of *StGA2ox2* or *StGA2ox4* but significantly decreased expression of *StGA2ox3*.

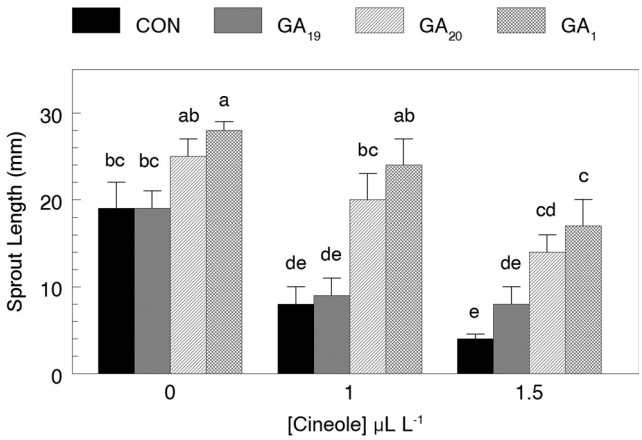

Fig. 6 Effects of GA_{19}, GA_{20}, and GA_1 on cineole-mediated inhibition of sprout growth. Minitubers were treated with 5 µL DMSO ± 10 µg GA_{19}, GA_{20}, or GA_1 and were then exposed to 1.0 or 1.5 µL L^{-1} cineole. After 14 days, final the length of the longest sprout per tuber was measured to the nearest mm. Data presented are means ± SE ($n = 10$). Means with the same letter are not significantly different at $P = 0.05$

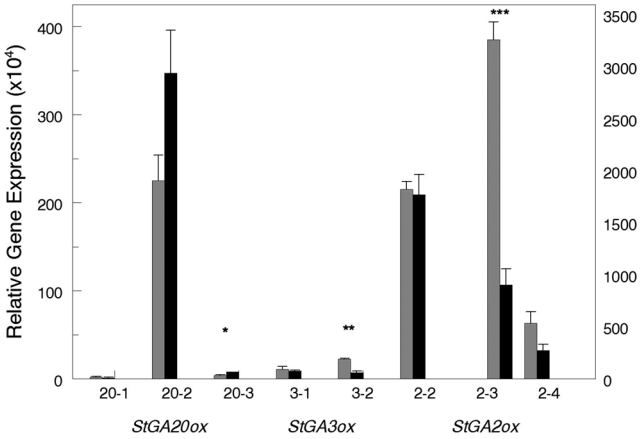

Fig. 7 Effects of cineole treatment on the expression of GA metabolic genes in tuber sprouts. Intact tubers were enclosed for 14 days in containers containing activated charcoal (control) or 1.5 µL L^{-1} cineole. qRT-PCR analysis was performed on RNA isolated from control and treated tissues. Relative transcript abundances were calculated by the $\Delta\Delta C_T$ method using the mean of C_T values for the two housekeeping genes (EF1α and actin) as normalizing factors. Right y axis: relative expression of *StGA2ox*-3; left y axis: relative expression of all other genes. Data presented are means ± SE ($n = 3$). *, **, *** indicate significant differences from controls at $P < 0.05$. 0.01, 0.001, respectively

Discussion

Because of the deleterious effects on the nutritional and processing qualities of potatoes, control of tuber sprouting is an essential component of successful potato storage management. With the exception of potatoes intended for the 'organic' market, postharvest sprout suppression is accomplished through the use of chemical sprout control agents (Kleinkopf et al. 2003). Most commercially used sprout-control agents act by either a herbicidal or phytotoxic mechanism. The most widely used sprout control agent, chlorpropham or CIPC, is a mitotic poison that disrupts microtubule function while clove oil, carvone, and α,ß-unsaturated carbonyls such as *trans*-3-decen-2-one and *trans*-3-nonen-2-one physically damage tuber sprout meristems (Knowles and Knowles 2012; Suttle et al. 2015). Of the registered compounds, only 1.4-dimethylnaphthalene appears to act in a non-phytotoxic and potentially reversible manner (Campbell et al. 2012).

Cyclic monoterpenes elicit a wide-range of biological activities and have been shown to inhibit growth in both bacterial and eukaryotic organisms (Sikkema et al. 1995). The growth-inhibiting properties of cineole were first described in studies examining the chemical nature of allelopathy in *Salvia leucophylla* (Muller et al. 1964). Like other terpenes, exposure of potatoes to high concentrations of cineole results in sprout tip necrosis and general cytotoxicity (Vaughn and Spencer 1991, 1993). At the concentrations used in these studies, necrosis was not observed in any plant tissue examined and the growth-inhibiting effects of cineole were quite selective. As evidenced by the lack of effect on root and callus

growth, cineole had no direct effect on cell division (Fig. 3). Further, the lack of cineole effect on seedling root growth and seed germination demonstrate no direct effect on the process of cell elongation. Of the assays examined, only cineole-mediated inhibition of etiolated seedling hypocotyl growth mirrored sprout growth inhibition. These observations are inconsistent with any form of general toxicity and suggest a more nuanced physiological action.

Hypocotyl elongation is a GA-mediated process and is the basis for one of the most widely used GA bioassays (Franklin and Wareing 1960). Similarly, sprout growth is dependent on GA synthesis and action as evidenced by the opposing effects of exogenous GA and GA deficiency on sprout length (Suttle 2004). In principle, cineole inhibition of sprout growth could be a result of impaired GA signaling, metabolism, or both. The ability of exogenous GA to reverse cineole-mediated sprout growth inhibition is not consistent with an effect on GA perception and action. The hypothesis that, at low treatment levels, cineole interferes with GA metabolism was explored using complementary approaches; both of which suggest that inhibition of the conversion of GA_{19} to GA_{20} plays a central role in the growth-inhibiting effects of low concentrations of cineole. This reaction is catalyzed by the enzyme GA-20 oxidase, a member of the 2-oxoglutarate-dependent dioxygenase family (Yamaguchi 2008; Kawai et al. 2014).

Interestingly although three separate reactions in the latter stages of GA biosynthesis ($GA_{53} \rightarrow GA_{44} \rightarrow GA_{19} \rightarrow GA_{20}$) are catalyzed by this enzyme, cineole treatment differentially affects only the last step. Prohexadione and daminozide, two known inhibitors of 2-oxoglutarate-dependent dioxygenases, inhibit the growth of many plants by blocking the biosynthesis of GA_1 but result in the accumulation of GA_{20} not GA_{19} as seen in cineole-treated spouts (Nakayama et al. 1992; Rademacher 2000). Unlike cineole, both of these inhibitors act competitively with the co-substrate 2-oxoglutarate which they structurally resemble (Brown, et al. 1997). Due to structural dissimilarity, it is unlikely that cineole acts in a similar manner and may inhibit only the final oxidation step.

Alternatively, it is possible that cineole has no direct effect on the catalytic activities of GA-20 oxidases and reduces GA content through an indirect mechanism such as gene expression or translation. To investigate this possibility, the effects of cineole treatment on the expression of two GA biosynthetic genes *(StGA20ox* and *StGA3ox*) and the principal catabolic gene (*StGA2ox*) were determined in sprout tissue. Cineole treatment had either small or insignificant effects on expression of genes encoding the two biosynthetic enzymes GA-20 oxidase and GA-3 oxidase. However, cineole treatment dramatically reduced expression of *StGA2ox-3*, the most abundantly expressed gene encoding the catabolic enzyme GA-2 oxidase (Fig. 7).

While this may seem paradoxical to the effects of cineole on GA content, these results likely reflect the nature of transcriptional control of GA metabolic genes. Previous studies have demonstrated that transcription of individual GA-related gene family members is differentially regulated in both a feed-forward and feedback manner (Yamaguchi 2008). In particular, genes encoding GA catabolic enzymes are often down-regulated in plants with reduced GA content. Thus, the effects of cineole treatment on the expression of GA metabolic genes may reflect a reduced content of bioactive GA, as suggested by the results in Fig. 6. This suggests that the reduction in GA content in following cineole treatment is a post-transcriptional effect.

Interestingly, the effects of registered growth retardants that interfere with GA biosynthesis on postharvest tuber sprout growth have received scant attention. Growth retardants have been widely used to stimulate in vitro tuberization but there have been few reports describing the sprouting behavior of these microtubers (Coleman et al. 2001). Sĭmko (1994) reported that inclusion of the GA biosynthesis inhibitor paclobutrazol in in vitro microtuber induction media increased tuberization and delayed tuber sprouting and reduced sprout elongation after 250 days of ex vitro storage. These results, together with those presented in this paper, suggest that growth retardants may represent a previously untapped source of potential sprout inhibitors for use in commercial settings.

In summary, the results presented in this paper indicate that the reversible inhibition of tuber sprout growth by low concentrations of cineole is primarily a result of reduced levels of bioactive GA. The reduction in GA content is not a result of altered expression of GA metabolic genes but may be due to differential inhibition of GA-20 oxidase activity blocking the conversion of GA_{19} to GA_{20}. It is important to note that these conclusions only apply to low concentrations of cineole that cause GA-reversible sprout growth inhibition. At higher cineole concentrations, sprout growth inhibition is only partially reversible and other more deleterious biochemical mechanisms are likely responsible.

References

Baydar, H., and T. Karadogan. 2004. The effects of volatile oils on in vitro potato sprout growth. *Potato Research* 46: 1–8.

Brown, R.G.S., H. Kawaide, Y. Yang, W. Rademacher, and Y. Kamiya. 1997. Daminozide and prohexadione have similar modes of action as inhibitors of the late stages of gibberellin metabolism. *Physiologia Plantarum* 101: 309–313.

Burton, W.G. 1989. *The Potato 3rd Edition*, 470–504. Essex: Longman Scientific & Technical.

Burton, W.G., and D.F. Meigh. 1971. The production of growth-suppressing volatile substances by stored potato tubers. *Potato Research* 14: 96–101.

Campbell, M.A., A. Gleichsner, L. Hilldorfer, D. Horvath, and J. Suttle. 2012. The sprout inhibitor 1,4-dimethylnaphthalene induces the

expression of the cell cycle inhibitors KRP1 and KRP2 in potatoes. *Functional and Integrative Genomics* 12: 533–541.

Chiwocha, S.D.S., S.R. Abrams, S.J. Ambrose, A.J. Cutler, M. Loewen, A.R.S. Ross, and A.R. Kermode. 2003. A method for profiling classes of plant hormones and their metabolites using liquid chromatography-electrospray ionization tandem mass spectrometry: analysis of hormone regulation of thermodormancy of lettuce (*Lactuca sativa* L.) seeds. *Plant Journal* 3: 405–417.

Coleman, W.K., D.J. Donnelly, and S.E. Coleman. 2001. Potato microtubers as research tools: a review. *American Journal of Potato Research* 78: 47–55.

Crowell, P.L., Z. Ren, S. Lin, E. Vedejs, and M.N. Gould. 1994. Structure-activity relationships among monoterpene inhibitors of protein isoprenylation and cell proliferation. *Biochemical Pharmacology* 47: 1405–1415.

Franklin, B., and P.F. Wareing. 1960. Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. *Nature* 185: 255–256.

Jones, M.G., R. Horgan, and M.A. Hall. 1988. Endogenous gibberellins in the potato *(Solanum tuberosum*). *Phytochemistry* 27: 7–10.

Kawai, Y., E. Ono, and M. Mizutani. 2014. Evolution and diversity of the 2-oxoglutarate-dependent dioxygenase superfamily in plants. *Plant Journal* 78: 328–343.

Kleinkopf, G.E., N.A. Oberg, and N.L. Olsen. 2003. Sprout inhibition in storage: current status, new chemistries and natural compounds. *American Journal of Potato Research* 80: 317–327.

Knowles, L.O., and N.R. Knowles. 2012. Toxicity and metabolism of exogenous α, ß-unsaturated carbonyls in potato (Solanum tuberosum L.) tubers. *Journal of Agricultural and Food Chemistry* 60: 11173–11181.

Meigh, D.F. 1969. Suppression of sprouting in stored potatoes by volatile organic compounds. *Journal of the Science of Food and Agriculture* 20: 160–164.

Meigh, D.F., A. Authur, E. Filmer, and R. Self. 1973. Growth-inhibitory volatile aromatic compounds produced by Solanum tuberosum tubers. *Phytochemistry* 12: 987–993.

Moreland, D.E. 1980. Mechanisms of action of herbicides. *Annual Review of Plant Physiology* 31: 597–638.

Muller, C.H., W.H. Muller, and B.L. Haines. 1964. Volatile growth inhibitors produced by aromatic shrubs. *Science* 143: 471–473.

Nakayama, I., M. Kobayashi, Y. Kamiya, H. Abe, and A. Sakuai. 1992. Effects of a plant-growth regulator, prohexadione-calcium (BX-112), on the endogenous levels of gibberellins in rice. *Plant & Cell Physiology* 33: 59–62.

Rademacher, W. 2000. Growth retardants: effects on gibberellin biosynthesis and other metabolic pathways. *Annual Review of Plant Physiology and Plant Molecular Biology* 51: 501–532.

Romagni, J.G., S.N. Allen, and F.E. Dayan. 2000. Allelopathic effects of volatile cineoles on two weedy plant species. *Journal of Chemical Ecology* 26: 303–313.

Sikkema J, JAM deBont, and B Poolman. 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiological Reviews 59:201–222.

Sĭmko, I. 1994. Effect of paclobutrazol on *in vitro* formation of microtubers and their sprouting after storage. *Biologia Plantarum* 36: 15–20.

Sponsel, V.M., and P. Heddon. 2004. Gibberellin biosynthesis and inactivation. In *In plant hormones: biosynthesis, signal transduction, action!* ed. P.J. Davies, 63–94. Dordrecht: Kluwer.

Suttle, J.C. 2004. Involvement of endogenous gibberellins in potato tuber dormancy and early sprout growth: a critical assessment. *Journal of Plant Physiology* 161: 157–164.

Suttle, J.C., L.L. Huckle, S. Lu, and D.C. Knauber. 2014. Potato tuber cytokinin oxidase/dehydrogenase genes: biochemical properties, activity, and expression during tuber dormancy progression. *Journal of Plant Physiology* 171: 448–457.

Suttle JC, MA Campbell, and NL Olsen. 2015. Potato tuber dormancy and postharvest sprout control. In: Postharvest Ripening Physiology in Crops. Ed. S. Pareek. CRC Press, Boca Raton. In Press.

Teper-Bamnolker, P., N. Dudai, R. Fischer, E. Belausov, H. Zemach, O. Shoseyov, and D. Eshel. 2010. Mint essential oil can induce or inhibit potato sprouting by differential alteration of apical meristem. *Planta* 232: 179–186.

Tsai, C.-J., S.A. Harding, T.J. Tschaplinski, R.L. Lindroth, and Y. Yuan. 2006. Genome-wide analysis of the structural genes regulating defense phenylpropanoid metabolism in *Populus*. *New Phytologist* 172: 47–62.

Vaughn, S.F., and G.F. Spencer. 1991. Volatile monoterpenes inhibit potato tuber sprouting. *American Journal of Potato Research* 68: 821–831.

Vaughn, S.F., and G.F. Spencer. 1993. Volatile monoterpenes as potential parent structures for new herbicides. *Weed Science* 41: 114–119.

Yamaguchi, S. 2008. Gibberellin metabolism and its regulation. *Annual Review of Plant Biology* 59: 225–251.

Yu, V., S. Rakitin, V. Vladimirtseva, and L.N. Nikolaeva. 1978. Action of maleic hydrazide on RNA and protein synthesis in growing potato buds. *Fiziologiya Rastenii* 25: 223–229.