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Inhibitory Effect of a Rhizobitoxine Analog on Bud Growth after Release from Dormancy

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RICHARD H. ZIMMERMAN, M. LIEBERMAN, AND OLIVIA C. BROOME
Agricultural Research Service, United States Department of Agriculture, Beltsville Agricultural Research Center, Beltsville, Maryland 20705

ABSTRACT

Application of the ethoxy analog of rhizobitoxine (L-2-amino-4-[2'-aminoethoxy]-*trans*-3-butenoic acid), an inhibitor of ethylene biosynthesis, inhibited growth of apple, crabapple, and apricot buds released from dormancy by chilling or by treatment with benzyladenine. When tea crabapple (*Malus hupehensis* [Pamp.] Rehd.) buds were sprayed once with 8.8×10^{-3} M benzyladenine, ethylene production by the buds increased significantly 24 to 48 hours after benzyladenine treatment. Application of the rhizobitoxine analog to the buds at the time of benzyladenine treatment reduced ethylene evolution to the level of the controls for up to 2 weeks after treatment. Increase in bud weight was inhibited also but to a lesser extent. These data suggest that growth of buds is accompanied by ethylene production and that the inhibition of ethylene biosynthesis also inhibits bud growth. Since additional metabolic effects result from the action of the rhizobitoxine analog, no firm conclusions on its role can be drawn at this time.

Hormonal control of bud growth appears to involve the integrated interactions of various plant hormones. Benzyladenine has been shown to stimulate or accelerate release of buds from dormancy (2). The mode of action is assumed to relate to the function of cytokinins in stimulating cell division. Cytokinins are also known to stimulate C_2H_4 production in some plants (3, 4). With this in mind, we attempted to determine the involvement of C_2H_4 in bud development by the use of the ethoxy analog of rhizobitoxine (L-2-amino-4-[2'-aminoethoxy]-*trans*-3-butenoic acid), an inhibitor of C_2H_4 biosynthesis which is known to inhibit conversion of methionine to C_2H_4 (6, 8).

Preliminary trials with buds of apricots and 'Jonathan' apples showed that the rhizobitoxine analog inhibited growth of buds released from dormancy by chilling or by BA treatment. Experiments described herein were then conducted using buds of tea crabapple which had been activated by BA (1) to determine: (*a*) the amount of the rhizobitoxine analog needed to inhibit bud growth; (*b*) the effectiveness of this inhibitor at various times after bud activation; and (*c*) the effect of the rhizobitoxine analog on C_2H_4 production by activated buds.

MATERIALS AND METHODS

Twelve to 18-month-old seedling trees of tea crabapple (*Malus hupehensis* [Pamp.] Rehd.) grown in the greenhouse as described previously (1) were used in the experiments. In one experiment, trees were defoliated, moved into a root cellar in January, and chilled for 3 months. In all other experiments, seedlings were not moved from the greenhouse but were defoliated prior to treatment.

Spray application of BA and methionine was done as described previously (1) and the rhizobitoxine analog was dissolved in water and 1 to 2 μl applied directly to the buds with a microsyringe.

For measurement of C_2H_4 production, three buds were excised and placed directly into a 5-ml plastic syringe, the plunger was reinserted, and adjusted to the 3-ml mark. A serum tube stopper was then placed over the tip of the syringe. After 2 hr, a gas sample was collected by inserting a needle on a second syringe through the serum stopper into the first syringe. The plunger on the second syringe was withdrawn while simultaneously the plunger on the first was pushed in until its face contacted the buds. Ethylene in the sample was then determined by gas chromatography using an alumina column and a flame ionization detector (7).

Treatments were replicated three to 12 times within individual experiments and all experiments in which C_2H_4 was determined were run at least twice.

RESULTS

Both 250 and 375 μg/bud of the rhizobitoxine analog strongly inhibited bud growth on tea crabapple seedlings moved to the greenhouse after 3 months of chilling (Table I; Fig. 1). Inhibition was the same for single applications of these amounts and multiple applications of 125 μg giving the same total amount. Although shoots from buds receiving a single application of 125 μg (1 μl) rhizobitoxine analog were shorter 13 days after treatment, these shoots had overcome the inhibitory effects of the compound by day 40. A single spray application of 1.25 μg/μl rhizobitoxine analog had no effect on bud growth (data not shown). Application of BA immediately following application of 375 μg rhizobitoxine analog did not overcome the inhibition of bud growth caused by the latter compound.

When tea crabapple buds were activated with BA, application of rhizobitoxine analog to the buds up to 96 hr after BA treatment was as effective in halting bud development as application at the same time as BA treatment (Table II). Increasing the amount of rhizobitoxine analog applied per bud from 159 to 954 μg resulted in only slightly greater inhibition of bud growth 3 weeks after BA treatment except when the rhizobitoxine analog was applied immediately after BA treatment (Table II).

Since rhizobitoxine analog inhibits C_2H_4 production in several tissues as well as inhibiting the growth of tea crabapple buds, several experiments were conducted to determine if rhizobitoxine analog inhibits C_2H_4 production in developing tea crabapple buds. The data in Figures 2 and 3 are from one of these experiments, all of which gave similar results. Activation of the buds with a single spray of 8.8×10^{-3} M BA resulted in a significant increase in C_2H_4 production 24 to 48 hr after treatment (Fig. 2). Ethylene evolution continued to increase for about 1 week after treatment and remained significantly greater than the controls

Table I. *Effect of rhizobitoxine analog (rhiz.) on bud growth of tea crabapple seedlings treated after 3 months of chilling.* Twenty buds per treatment with three replications were used.

Treatment	Shoot length at different times after treatment 13 days[1]	40 days
	cm	cm
Control	2.5 a	3.7 a
Rhiz., 125 µg	1.0 b	3.6 a
Rhiz., 250 µg	0.6 b	1.1 b
Rhiz., 375 µg	0.5 b	1.2 b
Rhiz., 125 µg x 2	0.5 b	0.9 b
Rhiz., 125 µg x 3	0.4 b	0.4 b

[1]Mean separation in columns by Duncan's multiple range test, 5% level.

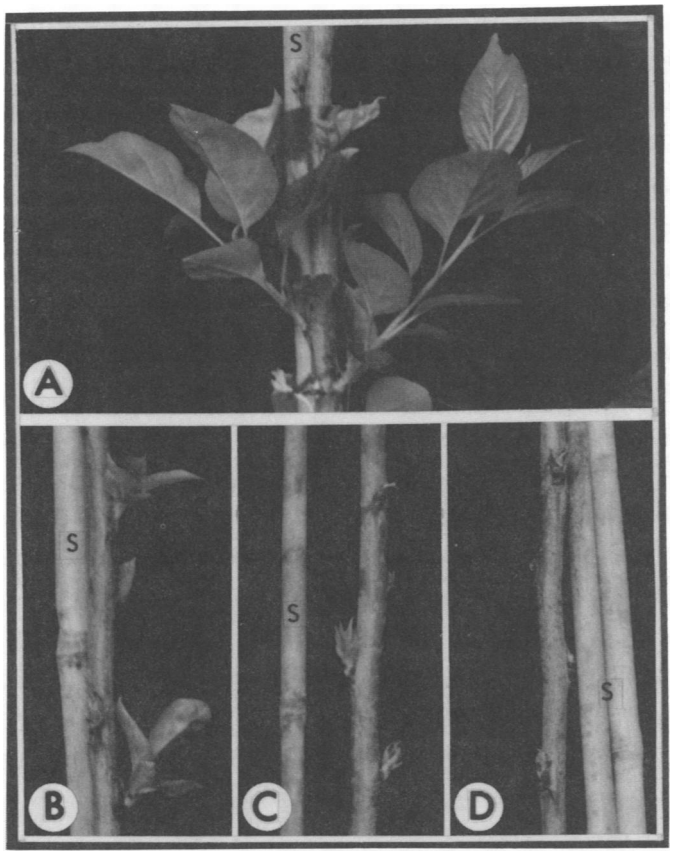

FIG. 1. Development of tea crabapple buds 15 days after treatment. Seedlings were defoliated and chilled for 3 months before treatment. A: Shoots from untreated buds; B: one; C: two; D: three applications of 125 µg rhizobitoxine analog. Individual applications for C and D were at daily intervals starting on the day the plants were returned to the greenhouse. Support stakes for the plants are indicated by S.

for the duration of the experiment. Control buds evolved a small quantity of C_2H_4 on each sampling date and this amount did not change appreciably during the course of the experiments. Application of 310 µg rhizobitoxine analog immediately following the BA treatment prevented any increase in C_2H_4 evolution above the control level for the duration of the experiment.

Fresh weight of buds treated with BA was greater than that of the controls by 3 days after treatment and remained so for the rest of the experiment (Fig. 3). The most rapid increase in fresh weight occurred after the 1st week. Weight of the control buds did not increase during the experiment. Buds treated with both BA and rhizobitoxine analog showed a steady but nonsignificant weight increase during the course of the experiment.

Table II. *Effect of amount and time of rhizobitoxine analog (rhiz.) application on growth of tea crabapple buds three weeks after activation with BA.* Benzyladenine (8.8×10^{-3}M) applied as a spray to runoff; rhizobitoxine analog (8.1×10^{-1}M) applied with a microsyringe, each 1 µl drop containing 159 µg. Three buds per treatment with ten replications were used. Bud development was rated on a scale of 1 (bud scales tight, no green showing, no swelling) to 8 (bud open, shoot elongating) (1).

Rhiz. applied per bud (µg)	Time of rhiz. application in hours after BA application 0	48	96	192	$\bar{x}$
0	6.0	5.9	5.7	6.5	6.0
159	5.1	3.9	4.2	4.9	4.5
318	3.7	3.8	3.6	5.5	4.2
954	3.2	3.2	3.5	5.5	3.9
$\bar{x}$	4.5	4.2	4.3	5.6	4.6

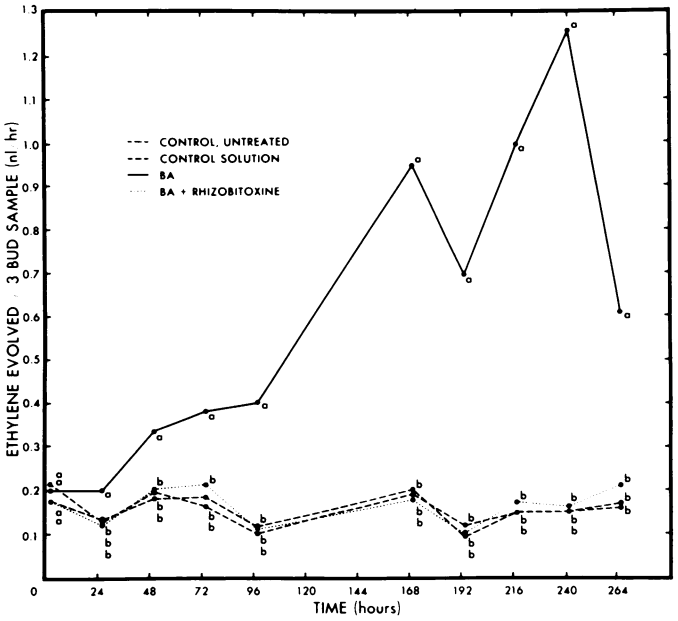

FIG. 2. Effect of BA and rhizobitoxine analog on C_2H_4 evolution by tea crabapple buds. A single spray application of 8.8×10^{-3} M BA was followed immediately by 310 µg of rhizobitoxine analog in a 2-µl drop. Control solution containing 1% Tween 20 and 5% dimethylsulfoxide was solvent for BA and was applied as a spray. Each data point is the mean of five replications. Mean separation on each sampling date by Duncan's multiple range test, 5% level.

An attempt to overcome the inhibition of bud break caused by rhizobitoxine analog was made using methionine which is a precursor of ethylene (5). Methionine did not counteract the effect of the inhibitor and, in combination with BA, did not stimulate bud growth (Table III). This suggests that rhizobitoxine analog blocks conversion of methionine to ethylene as previously shown (8).

To check the effect of C_2H_4 on bud break, Ethephon at a range of concentrations from 50 to 1000 µl/l and C_2H_4 gas dissolved in water at concentrations of 5 to 25 µl/l were sprayed on tea crabapple buds. No bud break was stimulated at any of the concentrations tested.

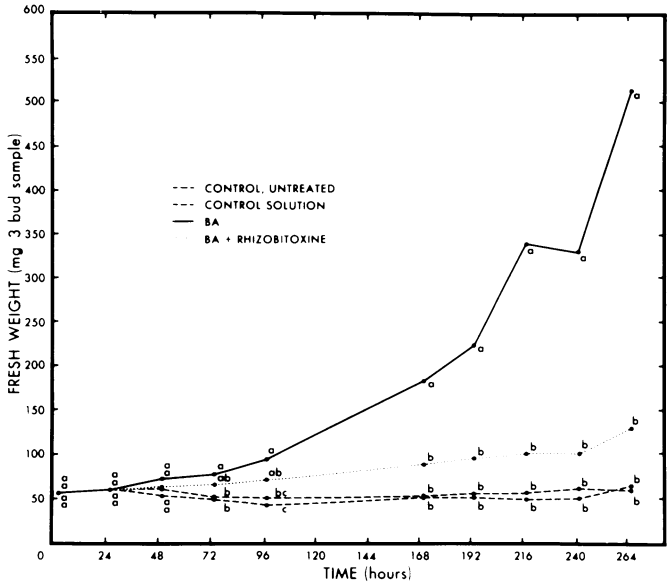

FIG. 3. Effect of BA and rhizobitoxine analog on fresh weight of tea crabapple buds. A single spray application of 8.8×10^{-3} M BA was followed immediately by 310 μg of rhizobitoxine analog in a 2-μl drop. Control solution containing 1% Tween 20 and 5% dimethylsulfoxide was solvent for BA and was applied as a spray. Each data point is the mean of five replications. Mean separation on each sampling date by Duncan's multiple range test, 5% level.

DISCUSSION

These studies show that rhizobitoxine analog inhibits growth of tea crabapple buds. Because rhizobitoxine analog is known to be an inhibitor of C_2H_4 biosynthesis in plants, it is reasonable to suspect that C_2H_4 might play a role in development of the bud after its release from dormancy (Fig. 2). Ethylene production increased significantly, relative to controls, after treatment with BA, and C_2H_4 production was suppressed when BA treatment was immediately followed by treatment with rhizobitoxine analog. In a parallel manner, growth of buds increased after BA treatment and was suppressed significantly when BA treatment was immediately followed by treatment with rhizobitoxine analog (Fig. 3).

These results suggest that bud break is followed by, rather than caused by, C_2H_4 production, since it was not possible to break bud dormancy by addition of C_2H_4 or Ethephon to dormant buds. These results contrast with those obtained with black currant buds which were forced into growth following treatment with C_2H_4 gas (9). Apparently, C_2H_4 action is not involved in breaking of dormancy of tea crabapple buds but may play a role in the initial stages of bud growth and development after dormancy is broken. The rise in C_2H_4 production by buds is observed 24 to 48 hr after BA treatment. Ethylene production by BA-activated buds is not an artifact of cytokinin treatment because buds induced to grow by cutting back tea crabapple seedlings produced C_2H_4 in amounts comparable to that produced by the BA-activated buds (Fig. 2).

Alternatively, the inhibitory action of rhizobitoxine analog on buds may be associated with the structural similarity of the inhibitor to methionine or other amino acids. We can postulate that the negative growth effect observed with rhizobitoxine analog resulted from its interference with amino acid metabolism, more specifically, with metabolism of methionine. However, the inhibitory action of rhizobitoxine analog on growth was not overcome by the addition of methionine. Possibly, the methionine did not penetrate the buds and so could not overcome rhizobitoxine analog inhibition. This is considered unlikely because of the similarity of structure between methionine and rhizobitoxine analog. Penetration of rhizobitoxine analog is assumed because of the response obtained. The inhibitor apparently did not produce a deficiency of methionine but acted by inhibiting the conversion of methionine to C_2H_4 (8). These experiments do not rule out additional effects of rhizobitoxine analog on methionine metabolism that may account for its inhibition of bud break and development. The results reported herein are consistent with the postulate that rhizobitoxine analog acts by suppressing C_2H_4 production after dormancy is broken by BA. They further suggest a role for C_2H_4 in growth and development in the period following bud break.

Table III. *Effect of single application of BA, rhizobitoxine analog (rhiz.) and methionine on growth of tea crabapple buds three weeks after treatment.* Methionine (5×10^{-3}M) and BA (8.8×10^{-3}M) applied as a spray to runoff; rhizobitoxine analog (7.9×10^{-1}M) applied as a 2 µl drop (containing 310 µg) with a microsyringe. Eight buds per treatment with 12 replications were used. Bud development was rated on a scale of 1 (bud scales tight, no green showing, no swelling) to 8 (bud open, shoot elongating).

Treatment	Rating[1]
Control	1.1 c
Methionine	1.2 c
BA	5.3 a
BA + methionine	5.2 a
BA + rhiz.	3.8 b
BA + rhiz. + methionine	3.5 b

[1]Mean separation by Duncan's multiple range test, 1% level.

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