Delaying Natural Flowering in Pineapple Through Foliar Application of Aviglycine, an Inhibitor of Ethylene Biosynthesis

Ren-Huang Wang and Yu-Mei Hsu

Kaohsiung District Agricultural Research and Extension Station, Pingtung, Taiwan, R.O.C.

Duane P. Bartholomew

Department of Tropical Plant and Soil Science, University of Hawaii, Manoa, HI 96822

Subbiyan Maruthasalam and Chin-Ho Lin¹

Department of Life Sciences, National Chung Hsing University, 250 Kuo-Kuang Road, Taichung 402, Taiwan, R.O.C.

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Abstract. In Taiwan, the major yield constraint in pineapple cultivation is natural flowering, which occurs when daylengths are shorter and nights are cooler. This natural (precocious) flowering increases the cost of cultivation and reduces the percentage of fruits of marketable size. Two field experiments were conducted to evaluate the inhibitory potential of aviglycine [(S)-trans-2-amino-4-(2 aminoethoxy)-3-butenic acid hydrochloride, AVG] on natural flowering of ‘Tainon 17’ pineapple plants during the 2003 to 2004 and 2004 to 2005 cropping seasons. In the 2003 to 2004 season, bolting in the control exceeded 80% on 2 Mar. 2004, whereas no bolting was observed in the treatments. Inhibition of bolting by aviglycine (AVG) was dependent on the concentration and frequency of application. Bolting was less than 40% when plants were treated in Nov. and Dec. 2003 with 500 mg·L⁻¹ of AVG four times at 15-day intervals or with five applications made at 10-day intervals. For the 2004 to 2005 season, bolting of plants treated with 250 or 375 mg·L⁻¹ AVG was delayed 4 weeks relative to the control, whereas bolting was delayed 7 weeks by four or five applications of 500 mg·L⁻¹ of AVG applied at 10- or 15-day intervals. Both experiments showed that four to five applications of 500 mg·L⁻¹ of AVG at 10- or 15-day intervals delayed inflorescence emergence relative to the control for the duration of the treatments. We assume control was maintained for 1 to 2 weeks after treatments stopped. Based on these results, the date AVG treatments stop can be used to estimate the duration of delay in flowering. AVG inhibits ethylene biosynthesis and has the potential to be effectively used to delay or completely control the problem of precocious flowering and associated crop losses in pineapple.

Pineapple (*Ananas comosus* var. *comosus*) (Coppens d’Eeckenbrugge and Leal, 2003) is an important fruiting monocot and produces the only edible fruit in plants of the family Bromeliaceae. A major limitation that afflicts pineapple growers is the phenomenon of natural flowering, which results in unscheduled fruiting. The percentage of natural induction is highly unpredictable and the incidence may vary from 0% to 100% in any given year (Kuan et al., 2005), which causes serious scheduling problems for growers and, in particular, fresh market growers. Fruits produced by natural induction may not find a market, are costly to harvest because a relatively small percentage of these fruits mature at any given time in a field, and sometimes

the fruits are too small to be marketable. These factors ultimately increase the cost of cultivation and reduce profits.

Being a tropical fruit, pineapple is highly susceptible to frost and is commonly planted within 30°N and S latitude. Natural flower induction in subtropical latitudes mainly occurs in cooler winter months, and in N latitudes typically occurs from late November through at least February, when temperatures are cooler and winter daylengths are shorter (Bartholomew et al., 2003). Van Overbeek and Cruzado (1948) provided the first evidence that low night temperature could enhance the induction of reproductive development in pineapple. However, the duration of the period of natural induction in a field of pineapples is determined by a variety of factors, including cultivar sensitivity, plant size, and the prevailing weather conditions, particularly temperature (Kuan et al., 2005; Lin et al., 2005).

Forcing (induction of flowering by external application of ethylene or related com-

pounds) of pineapple is a well-established commercial practice whereby pineapple can be induced to flower at any time of a particular year (almost irrespective of the temperature) and the fruits can be made available through nearly all of the year. Forcing efficiency will be high when forcing is done during the period when natural induction normally occurs. Forcing of pineapple is commonly done with ethylene, ethylene-releasing compounds such as ethephon, and CaC₂, or acetylene when the plants are of sufficient size so as to yield fruits of marketable value (Bartholomew et al., 2003). Forcing also synchronizes flowering and produces a sharp ripening peak. In Taiwan (latitude 22° to 26°N), natural induction begins in late November as a result of sudden drops in temperature that coincide with passing cold fronts. Hence, most forcing is done during September and October and the fruits are harvested from March to May of the next year. If forcing of fully developed plants is delayed beyond October, they are highly prone to natural induction in the following months (Kuan et al., 2005).

Management of flowering of pineapple can be controlled to some extent by cultural practices that reduce plant stresses and promote vegetative growth (Bartholomew et al., 2003). Alternatively, growers can inhibit natural induction during susceptible periods by spraying a plant growth regulator and flowering can later be forced by spraying ethylene or ethephon. A number of plant growth regulators will inhibit flowering of pineapple under field conditions. Gowing and Leeper (1960) reported that low concentrations of certain phenoxyacetic acids forced pineapple, whereas higher concentrations inhibited it. Others (Martinez et al., 1997, 2000; Scott, 1993) found that foliar sprays of 2-(3-chlorophenoxy)-propionic acid (CPA) reduced the percentage of natural induction of pineapple. Cunha et al. (2003) tested the effect of many growth regulators on natural flowering of pineapple and found that CPA and paclobutrazole inhibited natural flowering of pineapple at concentrations of 90 and 240 mg·L⁻¹, respectively. Kuan et al. (2005) reported that aviglycine (AVG) prevented natural flowering in ‘Tainon 18’ pineapple under field conditions. AVG is an inhibitor of ethylene biosynthesis that is registered in the United States to improve fruit retention of apples and pears. Recently, Martinez et al. (2005) reported that Fruitone CPA (20 mg·L⁻¹ of a.i.) produced more than 90% inhibition of natural flowering in the pineapple hybrid 73–114, which is also widely known as MD-2 (Chan et al., 2003), with four to six applications at weekly intervals starting from the last week of October or first week of November.

Although growth regulators can inhibit natural induction of pineapple, their practical application in pineapple fields has been limited by availability, cost, or both. The potential of AVG to control flowering in pineapple (Kuan et al., 2005) accompanied with the recent availability of large quantities of a commercial formulation of AVG has

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¹To whom reprint requests should be addressed; e-mail chinho@dragon.nchu.edu.tw

made it an attractive candidate for the control of a long-term problem in pineapple cultivation. Although Kuan et al. (2005) demonstrated that AVG will control pineapple flowering, the potential of AVG was not fully explored. The present study reports a more detailed investigation on the potential use of AVG for flowering control so that the grower can determine the date of initiation of reproductive development rather than depend on weather factors for this purpose.

Material and Methods

The current study was conducted in two cropping seasons (2003 to 2004 and 2004 to 2005) as two different experiments. The plant material used throughout this study was 'Tainon 17' ('Smooth Cayenne' × 'Rough') pineapple. The cultivar 'Tainon 17' was selected because of its popularity among the farmers of Taiwan (more than 70% of the pineapple area is occupied by 'Tainon 17') and its high tendency toward natural induction during winter months.

Expt. 1. The first experiment was carried out in a production field on the Lao-pei farm of the Taiwan Pineapple Corporation (22°N latitude). The experiment was initiated on 10 Nov. 2003 and the plants were treated with 5.0 ml of a solution containing 500 mg·L⁻¹ AVG (sold commercially as ReTain by Valent BioSciences Corp., Libertyville, IL), a water-soluble powder containing 15% a.i. supplemented with 0.02% Break-Thru (Goldschmidt Chemical Corp., Hopewell, VA), a nonionic surfactant. The details regarding the number of treatments and frequency of AVG application are given in Table 1. Plants treated with 5 ml of an aqueous solution containing 0.02% Break-Thru alone were kept as a control. Inflorescences emerged in the leaf whorl, defined as bolting, were recorded weekly beginning on 2 Mar. 2004 until a plateau was reached and the values are expressed as a percentage.

Expt. 2. The second experiment was conducted during the 2004 to 2005 production season on a private farm near the Lao-pei farm. Seven-month-old 'Tainon 17' plants were treated with four to five applications each of 5 ml of solutions containing 250, 375, or 500 mg·L⁻¹ of AVG and 0.02% Break-Thru at 10- or 15-d intervals between 10 Nov. and 25 Dec. 2004 (Table 2). Control plants were treated with 5 ml of a solution containing 0.02% Break-Thru and the percentage of bolting was recorded as described earlier.

Statistical analyses. In both experiments, ~30 plants were used for each treatment and each treatment was replicated at least four times. The field blocks were arranged in a randomized complete block design. The collected percentage data were subjected to arcsine transformation and statistically analyzed using SAS analysis of variance (SAS Institute, Cary, NC) and the means were ranked using Fisher's least significant difference test.

Results and Discussion

The bolting percentage for plants treated with AVG was significantly less than that of the control group at all assessment dates for the 2003 to 2004 cropping season. Moreover, all the natural induction had occurred in the control plants by 2 Mar. 2004, the date of the first assessment, because bolting did not increase thereafter (Fig. 1). The greatest degree of control over natural induction occurred with a treatment interval of 10 d, although at later assessment dates, the 10- and 15-d intervals were not significantly different. Bolting percentages for the 2×15D and 3×20D treatments were significantly higher than the

other AVG treatments in the latter part of March. The significantly higher bolting percentage in the 2×15D treatment relative to the 5×10D and 4×15D AVG treatments is assumed to be the result of flower induction that occurred attributable to early suspension of the treatments. Because both the 5×10D and 3×20D treatments ended on the same date, the higher bolting percentage in the latter treatment could be attributed to earlier recovery of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity after the treatments ended. Continued treatment of plants with AVG at 10- or 15-d intervals likely would continue the inhibition of natural induction. Treatments were suspended

Table 1. Aviglycine treatment date, frequency and dosage for the 2003 to 2004 cropping season.

Treatment ^a	Date (month/d) and concn (mg·L ⁻¹) of aviglycine					
	11/10	11/20	11/25	11/30	12/10	12/25
CK ^b	0	0	0	0	0	0
5×10D (A)	500	500	—	500	500	—
2×15D (B)	500	—	500	—	—	—
4×15D (C)	500	—	500	—	500	500
3×20D (D)	500	—	—	500	—	500

^aNo. of applications at the indicated interval (days).

^bFive milliliters of a solution containing 0.02% Break-Thru alone.

Table 2. Aviglycine treatment date, frequency and dosage for the 2004 to 2005 cropping season.

Treatment ^a	Date (month/d) and concn (mg·L ⁻¹) of aviglycine					
	11/10	11/20	11/25	11/30	12/10	12/25
CK ^b	0	0	0	0	0	0
5×10D (A)	250	250	—	250	250	—
4×15D (B)	250	—	250	—	250	—
5×10D (C)	375	375	—	375	375	375
4×15D (D)	375	—	375	—	375	—
5×10D (E)	500	500	—	500	500	500
4×15D (F)	500	—	500	—	500	500

^aNo. of applications at the indicated interval (days).

^bFive milliliters of a solution containing 0.02% Break-Thru alone.

Fig. 1. Percentage of bolting of pineapple plants treated between 10 Nov. and 25 Dec. 2003 (2003 to 2004 cropping season) with 0 (CK) or 500 mg·L⁻¹ aviglycine for the indicated number of times at 10-, 15-, or 20-d intervals.

at the end of December because previous results with 'Tainon 18' pineapple (Kuan et al., 2005) showed that stopping treatments at that time inhibited natural induction for at least another 60 d, the potential of plants to bolt decreases as the average temperature increases in February and March, and treatment with AVG is costly.

Based on the results obtained from the 2003 to 2004 cropping season, a second trial was conducted in 2004 to 2005 to verify the frequency and interval of effective AVG treatments in delaying flowering. AVG concentration was reduced in hopes of finding a more cost-effective treatment that would control natural induction. A gradual increase in bolting percentage was observed in the control treatment until it reached 100% on 22 Mar. 2005, 133 d after the initial treatment (Fig. 2). At that time, all treatments had significantly lower bolting percentages than the control. Bolting percentage decreased significantly as the AVG dosage was increased,

but there were few significant differences between 10- and 15-d application intervals at the same AVG concentration. Bolting percentage at the different concentrations of AVG increased over time, but it was less as the AVG concentration was increased. Bolting percentage for the 250 mg·L⁻¹ AVG treatment was similar to the control at 169 d after treatment. The maximum bolting percentage for the 375 and 500 mg·L⁻¹ AVG treatments remained significantly less than the control for the duration of the experiment, but differences were small for all but the 500-mg·L⁻¹ treatment applied at a 15-d interval (Fig. 2). Either that treatment serves to indicate the variable nature of natural induction in pineapple or was the result of a lucky coincidence in timing of AVG applications just before the occurrence of natural induction. Differences in bolting percentage among treatments were small at the end of the study (Table 3), but a pineapple grower intent on managing flower induction would,

depending on the farm schedule, either continue treatment beyond December or force flowering to achieve a planned result.

AVG-treated plants showed phytotoxic symptoms on young leaves with conspicuous yellowing. The degree of symptom increased with the frequency of AVG application and increase in AVG concentration. Most of the plants treated with 500 mg·L⁻¹ AVG took ≈1 month to recover, whereas some of the plants with severe symptoms of yellowing took more than 1 month to recover. No phytotoxicity was observed on pineapple plants treated with 0.02% Break-Thru alone. The role of phytotoxicity-induced ethylene production on flowering induction warrants further investigation.

The flowering induction of 'Tainon 17' in the 2003 to 2004 crop season occurred after 10 Dec. 2003. The data show that treating plants with 500 mg·L⁻¹ of AVG beginning on 10 Nov. with four applications at 15-d intervals would be sufficient to control flower induction resulting from low temperature from December to March of the following year. On 15 Dec. 2004, a cold front passed through the area where the experiments were conducted. AVG treatment on the day of the cold front did not effectively inhibit flowering in 'Tainon 17' pineapple (data not shown), possibly as a result of the accumulation effect conferred by endogenous ethylene biosynthesis.

In the 2004 to 2005 crop season, four consecutive treatments of 500 mg·L⁻¹ of AVG at 15-d intervals delayed inflorescence emergence by 7 weeks (Table 3; Fig. 2.), whereas the bolting was observed between 22 Feb. and 1 Mar. 2005 in the untreated plants. The delay in the onset of flowering of treated versus control plants could be accounted for by the 2 months of AVG treatments followed by a 1- to 2-week residual effect. Based on this fact, the final date to stop the AVG treatment can be used to determine the duration of delay in flowering.

The timing of inflorescence emergence (bolting) in pineapple depends primarily on the prevailing temperature (Malezieux et al., 1994) and occurs 60 to 75 d after floral induction begins. Any delay in flower induction resulting from AVG treatment delays bolting. AVG is a specific inhibitor of ACC synthase, which inhibits the plants' ability to synthesize ethylene and also its ability to initiate inflorescence development. However, the plants can recover their ability to synthesize ethylene some time after AVG treatment stops and, if conditions are favorable for induction, plants would also regain their ability to initiate inflorescence development.

Conclusion

In pineapple, the potential for natural induction under field condition declines as average air temperature increases. Thus, the need for control of natural induction in Taiwan is primarily between 1 Dec. and 28

Fig. 2. Percentage of bolting of pineapple plants treated between 10 Nov. and 25 Dec. 2004 (2004 to 2005 cropping season) with 0 (CK), 250 (A, B), 375 (C, D) or 500 mg·L⁻¹ (E, F) of aviglycine for the indicated number of times at 10- or 15-d intervals.

Table 3. Effect of aviglycine (AVG) treatment on pineapple bolting during 2004 to 2005 cropping season.

Treatment ^a	AVG concn (mg·L ⁻¹)	Date of bolting initiation	Week of 50% bolting	Date to reach maximum bolting rate	Maximum bolting rate (%)
CK ^b	0	8 Feb.	22 Feb. to 1 Mar.	22 Mar.	100.0 a
5×10D (A)	250	8 Mar.	22–29 Mar.	24 May	94.2 bc
4×15D (B)	250	15 Mar.	22–29 Mar.	26 Apr.	99.2 ab
5×10D (C)	375	22 Mar.	12–19 Apr.	24 May	92.5 c
4×15D (D)	375	15 Mar.	12–19 Apr.	24 May	92.5 c
5×10D (E)	500	22 Mar.	19–26 Apr.	24 May	91.7 c
4×15D (F)	500	29 Mar.	19–26 Apr.	24 May	85.8 c

In a column, means followed by a common letter are not significantly different at the 5% level by Fisher's least significant difference test.

^aNo. of applications at the indicated interval (days).

^bFive milliliters of a solution containing 0.02% Break-Thru alone.

Feb., at which time plants will no longer be exposed to cold fronts and average temperature will have increased to the point at which vegetative growth rate increases, reducing plant sensitivity to natural induction. Based on the two field trial results, we conclude that four to five consecutive applications of 500 mg·L⁻¹ AVG made at 10- or 15-d intervals starting in the second week of November will delay natural flowering up to 7 weeks in 'Tainon 17' pineapples. The application schedule optimized in this study could also be extended for controlling precocious flowering in other cultivars of pineapple.

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