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ORIGINAL PAPER

Forced flowering of pineapple (*Ananas comosus* cv. Tainon 17) in response to cold stress, ethephon and calcium carbide with or without activated charcoal

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Abstract Ethylene, a gaseous plant hormone, is responsible for the initiation of reproductive development in pineapple. Reproductive development can be forced in pineapple (*Ananas comosus* var. *comosus*) throughout the year with ethylene. Inhibition of natural flowering initiation with aviglycine [(*S*)-trans-2-amino-4-(2-aminoethoxy)-3-butenoic acid hydrochloride], an inhibitor of ethylene biosynthesis, provides evidence that reproductive development in response to cold stress and short daylength is also in response to ethylene production. We studied the effect of cold treatment of pineapple on ethylene production and flower induction by applying a short-term cold stress to stem apices. Shoot apices of pineapple treated with ice crystals also produced twice as much ethylene as did those of control plants and significantly more than was produced by "D" leaf basal tissue. Moreover, pineapple plants treated four times with ice crystals or ice water were induced to flower under field conditions and the forcing efficiency, as evaluated by the percentages of inflorescence emergence and fruit harvest, was comparable to forcing with calcium carbide (CaC_2) and ethephon. In another field experiment two applications of a 1.0% solution of CaC_2 or 0.15% ethephon applied at 48 h intervals was sufficient to force reproductive development of 'Tainon 17'. Furthermore, 0.5 or 1.0% solutions of CaC_2 supplemented with 0.5% activated charcoal (AC) significantly improved the forcing effectiveness of CaC_2. This could/would make it possible to reduce the number or concentration, or both, of CaC_2 required to effect forcing in pineapple.

Keywords Activated charcoal · Calcium carbide · Ethylene · Inflorescence emergence · Ice · Pineapple

Abbreviations

AC	Activated charcoal
DAT	Days after treatment
GC	Gas chromatography
LSD	Least significant difference

Introduction

Synchronization of flowering is an essential component of pineapple (*Ananas comosus* var. *comosus*) (Hepton 2003) cultivation, especially for those cultivars intended for fresh consumption. Natural flowering out of season can cause serious scheduling problems for growers. Induction of reproductive development in pineapple under natural conditions is favoured by shortened day-length and cool night temperatures (van Overbeek and Cruzado 1948a; Gowing 1961; Friend and Lydon 1979; Friend 1981); however, other stresses can also induce flowering (Bartholomew et al. 2003). 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 (Wang et al. 2007).

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Natural flowering induction in pineapple in Taiwan and other locations subjected to continental-climate cold fronts is assumed to be principally mediated by ethylene burst(s) in the shoot apical meristem due to lowered night temperatures during cooler winter months. Recent research has shown that natural flowering of 'Tainon 17' and 'Tainon 18' pineapple can be prevented with aviglycine ([*S*]-Trans-2-amino-4-(2-aminoethoxy)-3-butenoic acid hydrochloride, sold commercially as ReTain®), an inhibitor of ACC (1-aminocyclopropane-1-carboxylic acid) synthase activity and thereby ethylene biosynthesis (Kuan et al. 2005; Wang et al. 2007). However, other growth regulators have also been recommended for this purpose (da Cunha et al. 2003). Flowering can be achieved later, typically with ethylene, ethephon and acetylene-releasing calcium carbide (CaC_2) (Bartholomew et al. 2003). Thus, prevention of natural flowering and artificial forcing thereafter at convenience, are two very important agronomic practices involved in pineapple cultivation. Any effective forcing agent, however, should result in uniformity of the harvest peak and fruit yield so as to have a steady supply to the cannery and the fresh-fruit market. In general ethylene is the most effective flowering inducer, but in Taiwan, small and marginal farmers prefer CaC_2 as the principal forcing agent because of low cost and ease of application. In the absence of a good understanding of the physiology of pineapple flowering, a pineapple grower would tend to apply multiple sprays of CaC_2 or ethylene related compounds, or a combination of both, so as to ensure complete forcing. Such practices increase the cost of forcing and reduce the profit (personal communication from the grower).

The use of synthetic flowering inducers or ethylene for forcing of organically-cultivated pineapple plants is not allowed in some countries. In addition, the emerging public reluctance towards consumption of artificially induced agricultural produce highlight the need for a truly organic means of pineapple forcing. Although anecdotal comments indicated cold water has been used in organic pineapple culture to induce flowering of pineapple, no published studies were found on the fluctuations in endogenous ethylene levels upon cold stress and their subsequent effect on reproductive development of pineapple. However, van Overbeek and Cruzado (1948a) showed that the exposure of 'Red Spanish' pineapple to low temperature for an extended period could initiate reproductive development under controlled conditions.

Hence, the present study was conducted with the following objectives, (1) to measure the effect of ice or ice water treatment on ethylene production in the laboratory and on flowering of 'Tainon 17' pineapple under field conditions and compare it with the efficacy of CaC_2 and ethephon forcing, and (2) to improve the forcing effectiveness of CaC_2 by supplementing it with activated charcoal (AC) so as to facilitate uniform flowering and reduce the cost of forcing pineapple.

Materials and methods

The cultivar 'Tainon 17' [a cross between 'Smooth Cayenne' (♀) and 'Queen' (♂)] was used in all the experiments. This cultivar is highly sensitive to natural flowering during cooler winter months (Wang et al. 2007).

Effects of ice on ethylene production

About 500 g of ice crystals were placed at the centre of the rosette of greenhouse-grown 1-year-old pineapple plants and allowed to melt. Control plants were treated with 500 ml of tap water (25°C). After melting was complete (≈after 1 h), 1.0 cm^2 discs were excised from the shoot apex or basal white portion of a "D" leaf (defined as the youngest physiologically mature, fourth visible leaf from the shoot apex; Devadas 2005) and vacuum infiltrated for 5 min with an aqueous solution containing 100 μM cycloheximide. The tissues were then transferred into 25 ml Erlenmeyer flasks containing 1.0 ml of a buffer solution containing 2% sucrose (w/v), 50 μg ml^{-1} chloramphenicol, 50 mM MES [pH 6.1; 2-(*N*-morpholino)ethanesulfonic acid] and 100 μM cycloheximide and sealed with serum caps. At 1 h intervals, a 1.0 ml sample of gas was drawn from the flask head space and analyzed for ethylene (C_2H_4) using a gas chromatograph (Shimadzu GC-14A; Shimadzu Co., Kyoto, Japan) with an alumina packed column (30 mm I.D. × 2.5 m, stainless steel) and a flame ionization detector (FID), as described by Huang and Lin (2003). After every sampling, the flasks were opened for 1 min, flushed with fresh air for an additional 30 s with an air-pump and recapped. The experiment was repeated at least three times with three replicates in each test and the mean ethylene liberation was calculated. A known concentration (1.18 ppm) of ethylene gas was used as the standard.

Forcing of ice-treated 'Tainon 17' plants in the field

Field experiments to study forced flowering of pineapple in response to cold stress, ethephon and CaC_2 treatments were conducted in the 2006–2007 and 2007–2008 cropping seasons in a commercial pineapple field at Minsyong (located at 23.57°N, 120.34°E), Chiayi county, Taiwan. Treatments were applied to 11-month-old plants having a vegetative mass of ≈3.5–4.0 kg when the average night temperature dropped to 20 ± 1°C and, based on records for the previous year, 2–3 weeks prior to the natural induction.

Experiment 1

During the cropping season 2006–2007, field-established 'Tainon 17' plants were treated once or twice with ice crystals or ice water starting on 29 October 2006 (Table 1). The plants in each treatment group were treated as follows: T1. Plants were treated twice, at 24 h intervals with 500 ml of water (25°C); T2. Plants were treated twice, at 48 h intervals with 50 ml of 1.0% (w/v) aqueous solution of CaC_2; T3–T4. About 500 g of ice crystals were packed in nylon mesh (3–4 mm) and placed in the centre leaf rosette of plants, once or twice (2*X*) at 24 h intervals; T5–T6. About 500 ml of ice water was poured directly onto the rosette with a beaker, once or twice (2*X*) at 24 h intervals; T7. Plants were treated 4 times at 15 day intervals (4*X*15D; Wang et al. 2007) with 5 ml of 500 ppm aviglycine (ReTain®-15% a.i., Valent BioSciences Corp., Libertyville, IL) containing 0.02% Break-Thru (Goldschmidt Chemical Corp., Hopewell, VA). Treatments T1, T2 and T7 served as controls. Treatments were applied between 5 and 7 pm. Inflorescence (=bud) emergence (red heart stage; Coppens d'Eeckenbrugge and Leal 2003) at the centre of the leaf rosette was recorded at weekly intervals starting from 16 December 2006 until percentage emergence reached a plateau.

Experiment 2

During the 2007–2008 cropping season, field-grown 'Tainon 17' plants were treated 3 or 4 times with 500 g to 2 kg of ice crystals or 500 ml of ice water at 24 h intervals, to impose as much cold-stress as possible, starting on 18 October 2007. Ice or ice water treatments were followed as specified above. Plants treated twice at 48 h intervals with 50 ml of 1.0% (w/v) CaC_2 or 0.15% (v/v) ethephon (prepared from 39.5% 2-chloro-ethyl phosphonic acid, Bayer Thai Co., Ltd., Bangkok, Thailand) or water (25°C) served as controls. The details of different treatments are given in the legend for Fig. 2. All the treatments were applied between 5 and 7 pm. Inflorescence emergence was recorded at weekly intervals starting from 1 December 2007 until percentage emergence reached a plateau.

Measurement of acetylene liberation by GC and forcing with CaC_2 in the field

Measurement of acetylene (C_2H_2) liberation from 0.25 g of CaC_2 mixed with 0.25 to 2.0% (w/v) 200–400 mesh activated charcoal (AC; Sankyo, Japan) and 25 ml of water (25°C) to facilitate acetylene liberation was done in a 50 ml flask. After 1 h, 1.0 ml of gas was drawn from the flask head space and analyzed for acetylene as described above for ethylene measurement. Subsequent measurements were made at 2 h intervals. After each sampling, the flasks were opened for 1 min, flushed with fresh air for 30 s with an air-pump and recapped. The analysis was repeated three times with three replicates in each trial and the mean acetylene liberation was estimated. For comparison, acetylene liberation from CaC_2 in an equal volume of water was measured.

Table 1 Percentage of forced flowering of 'Tainon 17' pineapple after treatment with an aqueous solution of CaC_2, ice or ice water in the 2006–2007 cropping season

Treatments[a]	Inflorescence emergence (%)[b]					
	Date (days after treatment)					
	16 Dec. 2006 (49)	16 Jan. 2007 (81)	27 Feb. 2007 (123)	06 Mar. 2007 (131)	20 Mar. 2007 (145)	10 Apr. 2007 (166)
T1. Control (2*X*)	0	0	20.8	75.0	87.5	100
T2. 1.0% CaC_2 (2*X*)	100	100	100	100	100	100
T3. Ice-500 g (1*X*)	0	0	79.1	96.0	96.0	100
T4. Ice-500 g (2*X*)	0	0	87.5	91.6	96.0	100
T5. Ice water-500 ml (1*X*)	0	0	20.8	58.3	83.3	91.6
T6. Ice water-500 ml (2*X*)	0	0	70.8	96.0	100	100
T7. Aviglycine (4*X*15D)	0	0	0	0	20.8	100
$LSD_{0.05}$	Treatments (t)[a] = 2.1; date (d)[b] = 1.9; $t \times d = 5.2$					

'*X*' denotes the indicated number of applications of ice or ice water at 24 h, CaC_2 at 48 h and aviglycine at 15 day intervals

The percentage data were subjected to arcsine transformation followed by LSD test at $P = 0.05$

[a] Treatments were initiated on 29 October 2006 and each treatment had three replicates ($n = 24$)

[b] Flowering induction as indicated by inflorescence emergence in the leaf rosette was recorded weekly starting on 16 December 2006 (only selected values are shown)

To test the efficacy of CaC_2 combined with AC as a forcing material during the 2007–2008 cropping season, 'Tainon 17' plants in a field were treated once or twice at 48 h intervals with 50 ml of an aqueous solution containing 0.5 to 1.0% CaC_2 (w/v) and 0.5 to 2.0% (w/v) of AC between 5–7 pm (Fig. 5). Before treatment, the aqueous solution containing CaC_2 and AC was allowed to react for 20 min to facilitate acetylene absorption by the AC. The experiment was initiated on 18 October 2007. The percentage of inflorescence emergence was recorded weekly beginning on 1 December 2007 until a plateau was reached.

Statistical analyses

In all the field experiments, at least 24 plants were used for each treatment and each treatment was replicated three times in a randomized complete block design (RBD). The percentage data on inflorescence emergence and fruit harvest were subjected to arcsine transformation followed by least significant difference (LSD) test at $P = 0.05$ using IRRISTAT Version 3.1 (Biometrices Unit, International Rice Research Institute, Manila, The Philippines).

Results and discussion

Effects of ice on ethylene production

The shoot apex and "D" leaf bases excised from ice-treated plants produced more ethylene than did control tissues. Production was greater from the shoot apex than from "D" leaf bases (Fig. 1a) and production from the ice-treated apical tissue was twice that produced by apical tissue from control plants 4 h after excision (Fig. 1a). The results also show that apical tissue responded more quickly to the cold treatment than did the leaf basal tissue (Fig. 1a, b). Interference of wound-induced ethylene with endogenous ethylene presumably formed due to cold treatment was prevented by infiltrating tissues immediately after excision with 100 μM cycloheximide for 5 min. Cycloheximide is a potent inhibitor of protein synthesis which can suppress the induction of ACC synthase upon wounding (Hyodo et al. 1983).

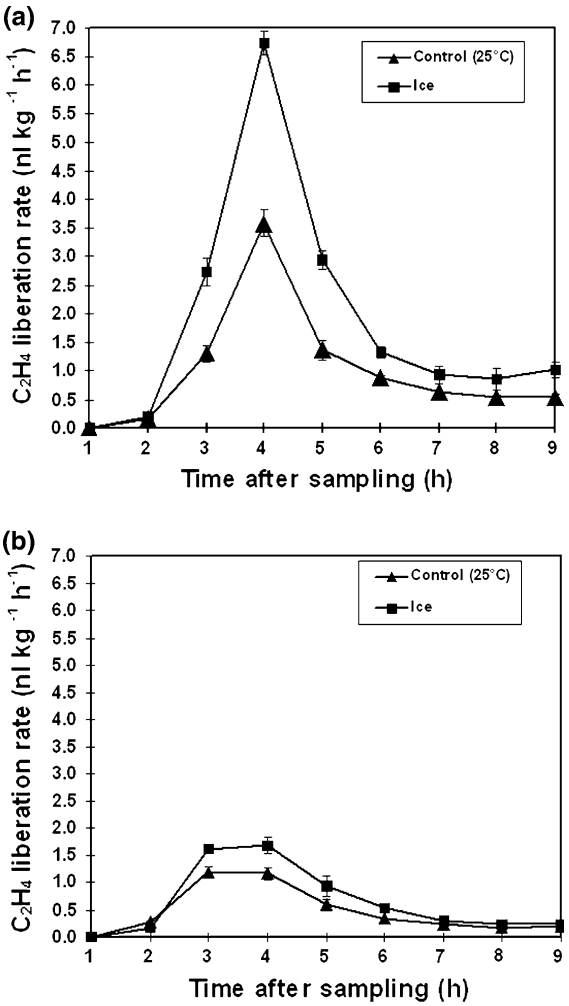

Fig. 1 Ethylene liberation from shoot apical (**a**) or "D" leaf basal tissue (**b**) of 'Tainon 17' pineapple treated with 500 g of ice or untreated. Each data point represents the mean ± SE of three replicates ($n = 3$)

Forcing of ice-treated 'Tainon 17' plants in the field

In the study conducted during 2006–2007, pineapple plants treated twice at 48 h intervals with 1.0% CaC_2 (T2) starting October 29 had 100% flowering induction at the time of first assessment on 16 December 2006 (Table 1). Inflorescence emergence for plants treated once or twice with ice crystals (T3–T4) or ice water (T5–T6) was not observed until the third week of February 2007. Soler et al. (2006) reported that forcing of pineapples grown on organic farms with 5°C water was not as successful as chemical-forced flowering. In our experiment, most plants in the ice treatments had a high percentage of inflorescence emergence on 27 Feb. 2007, 123 days after treatment (DAT) whereas inflorescence emergence for control plants (T1) was just over 20% on that date (Table 1). The delay in induction of the ice-treated plants when compared to CaC_2 treatment could be because the cold stress was insufficient to induce the ethylene levels required for forcing. By 166 DAT, inflorescence emergence was at or near 100% in most treatments (Table 1). Flowering induction of those plants without CaC_2 is assumed to be due their continued exposure to cool to cold temperatures and short daylengths during the months of December, January and February. These results indicate that 1–2 ice treatments neither promoted nor inhibited induction of flowering, but the plants'

sensitivity to natural induction in the following winter months was increased. Taken together GC data and field trial results we predict that the plants indeed produce higher levels of endogenous ethylene upon cold treatment. However, in the 2006–2007 season, ethylene production stimulated by ice treatment was not sufficient to force inflorescence development.

In the 2007–2008 cropping season, 90% of buds of plants forced with ethephon (2*X*, T3) had emerged within 51 days (Fig. 2) while plants treated with CaC_2 (2*X*, T2) or four times with ice or ice water emerged at 72 DAT (Fig. 2). At that time, no buds had emerged from 25°C water treated (T1) plants. At 93 DAT, the percentage induction for treatments T2, T3, and ice treatments T7 and T8 was over 90% while the T1 had only 4.0% flowering (Fig. 2). The reason for the reduced effectiveness of CaC_2 in this experiment is not known. Moreover, ice treated plants, comparing to chemical-forced plants, showed 2–3 week delayed inflorescence emergence (Fig. 2). The delayed response to the ice treatments could possibly be explained by the fact that they initiated bud development as a result of a cumulative effect whereas ethylene and acetylene are assumed to act directly.

Intriguingly, the 500 ml ice water (4*X*, T7) treatment gave better forcing than the same number of 500 g ice crystal (T5) treatments (Fig. 2). It is difficult to explain why the relatively longer ($\approx$1.0 h each day) cold-stress imposed on plants by melting ice crystals resulted in less induction than the transient cold-stress created by ice water. Studies of the temporal fluctuations in ACC synthase activity, the rate-limiting enzyme involved in ethylene biosynthesis (Kende 1993), after ice or ice water treatment would be needed to explain this interesting phenomenon.

The reason why the percentage of flowering induction was earlier and also higher with four treatments with ice crystals or ice water than with two likely can be inferred from the results of the GC analysis (Fig. 1a). Ethylene liberation from the stem apex reached a peak 4 h after sampling, i.e. 5 h after a 1 h ice treatment. It is likely that such peak occurs after every cold-stress, thus accumulating the level of ethylene exposure required to initiate the transition from vegetative to reproductive development.

More than 70%, and in some treatments, more than 90% of fruits were harvested from the CaC_2 (2*X*, T2) and ethephon (2*X*, T3) treated plants by the third week of May 2008, 216 DAT (Fig. 3). In most cases maturity and harvesting of fruits in the various treatments corresponded well to the pattern of inflorescence emergence (Fig. 2). Thus, fruit from plants forced with ice (T6, T7, and T8) were all harvested 2–3 weeks later than those from T2 and T3. In all the above treatments, fruit harvest was completed in 3–4 passes over a span of 7–10 days. Harvest of fruits from the forced plants was almost complete by the time water-treated plants were ready to harvest (Fig. 3). When plants were forced in October, cold stress was almost as effective as ethephon and CaC_2 and, most importantly, no observable morphological differences were seen among fruits from the various treatments (personal communication from the grower). Our next phase of study will focus on the qualitative analysis of fruits obtained from cold stressed plants.

The cultivar 'Tainon 17' used in this study is highly sensitive to low night temperature and therefore, can be forced with ice treatments with relative ease in October. As forcing efficiency is predominantly determined by cultivar sensitivity, the number of ice treatments required to get desired results may vary depending upon the cultivar being

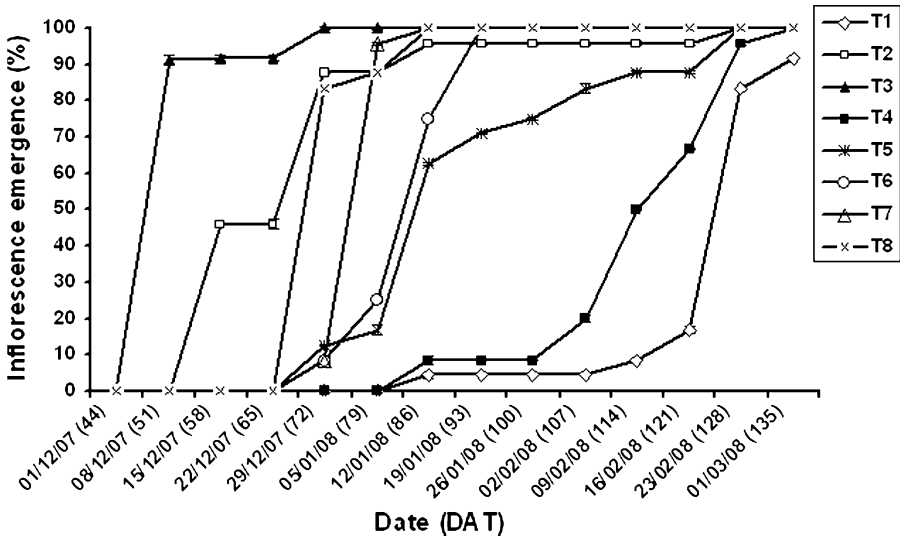

Fig. 2 Effect of chemical and cold stress treatments on forced flowering of 'Tainon 17' pineapple during the 2007–2008 season. The treatments were T1, water control (25°C); T2, 1.0% aqueous CaC_2 (2*X*); T3, 0.15% aqueous ethephon (2*X*); T4, 500 g ice (3*X*); T5, 500 g ice (4*X*); T6, 500 ml ice water (3*X*); T7, 500 ml ice water (4*X*); T8, ice 2 kg (4*X*). '*X*' denotes the indicated number of applications at 24 h, or in the case of CaC_2 and ethephon, 48 h intervals. Each data point represents the mean ± SE of three replicates ($n = 24$)

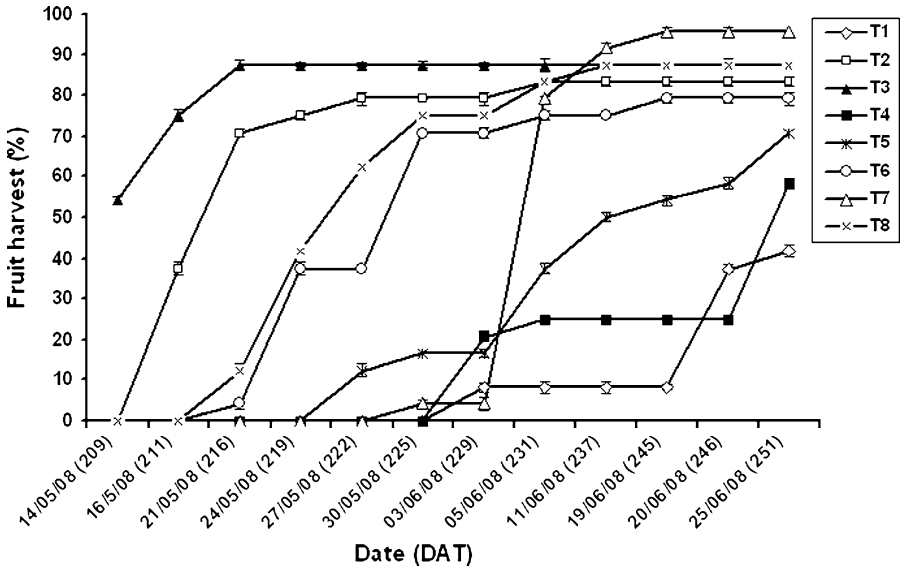

Fig. 3 Percentage of 'Tainon 17' fruit harvested after forcing with chemical or cold stress treatments during the 2007–2008 season. The treatments were T1, water control (25°C); T2, 1.0% aqueous CaC_2 (2*X*); T3, 0.15% aqueous ethephon (2*X*); T4, 500 g ice (3*X*); T5, 500 g ice (4*X*); T6, 500 ml ice water (3*X*); T7, 500 ml ice water (4*X*); T8, ice 2 kg (4*X*). '*X*' denotes the indicated number of applications at 24 h, or in the case of CaC_2 and ethephon, 48 h intervals. Each data point represents the mean ± SE of three replicates ($n = 24$)

forced, albeit several other factors also play a critical role (van Overbeek and Cruzado 1948b; Friend 1981; Py et al. 1984; Bartholomew et al. 2003). It is neither practical nor sustainable to apply multiple cold-shock treatments over large pineapple plantations; but plants grown organically in small acreages could be easily forced with ice or ice water. We believe that the organically-grown pineapple fruits would monetarily benefit growers by virtue of their higher market value, while the health conscious consumers would enjoy the benefit of organically-produced pineapples. In addition, our results also provide greater insight into the relationship between cold-stress and reproductive development in pineapple under natural conditions.

Measurement of acetylene liberation by GC and forcing with CaC_2 in the field

The addition of 0.5% AC to 1.0% CaC_2 delayed the release of acetylene from the solution (Fig. 4). Irrespective of the addition of AC, acetylene liberation peaked within 1 h after the start of the reaction. In the absence of AC, acetylene release declined to zero after 5 h while with AC, significant acetylene release continued even after 24 h (Fig. 4). Decreasing the concentration of AC below 0.5% did not significantly delay acetylene liberation relative to the control (data not shown). Preliminary GC analysis also showed no detectable acetylene release from samples with 1.0 or 2.0% AC (w/v) after about 5 h (data not shown). To test the significance of this delayed release of acetylene on forcing of pineapple, plants in a field were treated with CaC_2 alone and with added AC (0.5–2.0%) during the 2007–2008 cropping season. Both the number of applications and concentration of CaC_2 were reduced with the hope of finding a cost-effective combination of CaC_2 and AC.

The acetylene released when CaC_2 reacts with water is what actually forces pineapple to flower and it is equally as effective as ethylene (Bartholomew et al. 2003). Though the addition of AC to enhance the effect of ethylene forcing is a common practice (Py et al. 1987; Hepton 2003), the only published report found evaluating this practice shows it is ineffective (Van de Poel et al. 2009). However, two recent reports described the possible improvement of ethylene or acetylene action by supplementation with AC (Soler et al. 2006; Lin et al. 2009).

In our study, a mixture of a solution containing 1.0% CaC_2 and 0.5% AC applied once (T4) induced inflorescence emergence in 84% of plants, which was not-significantly ($P = 0.05$) different from 1.0% CaC_2 (2*X*, T2) treatment at 72 DAT (Fig. 5). On the same day of assessment, 1.0% CaC_2 (1*X*, T3), 1.0% CaC_2 + 1.0% AC (1*X*, T5) and 1.0% CaC_2 + 2.0% AC (1*X*, T6) treatments showed only 58, 54 and 29% bud emergence, respectively. Thus, the overall improvement in the forcing efficiency of

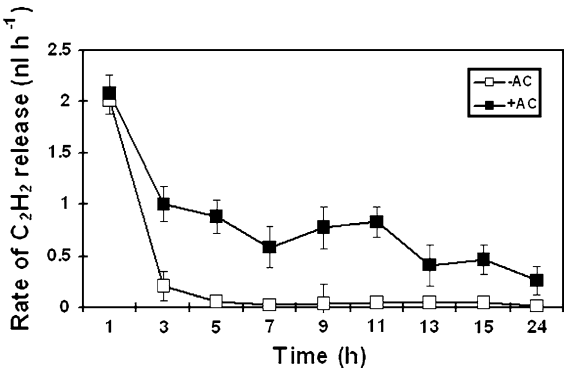

Fig. 4 Effect of the addition of 0.5% activated charcoal to 1.0% aqueous solution of CaC_2 on acetylene release. Each data point represents the mean ± SE of three replicates ($n = 3$)

CaC_2 by the addition of 0.5% (w/v) AC was found to be around 30 to 40%. These data clearly indicate that addition of 0.5% AC improved the effectiveness of CaC_2, while a higher concentration of AC actually reduced forcing, presumably due to a delayed or reduced release of acetylene gas trapped by high molar ratios of carbon molecules; this data confirms our previous report (Lin et al. 2009) and also is confirmed by preliminary GC analysis. In contrast to our findings, Van de Poel and De Proft (2009) reported that up to 5% activated carbon was required to increase ethylene absorption. These results are suggestive of possible variations occurring in the affinity of AC towards acetylene and ethylene.

Similarly, a mixture of 0.5% CaC_2 and 0.5% AC applied once (T9) resulted in a twofold higher flowering rate than 0.5% CaC_2 (2*X*, T7) and 0.5% CaC_2 (1*X*, T8) treatments, but none of them were as effective as 1.0% CaC_2 (2*X*, T2) and 1.0% CaC_2 + 0.5% AC (1*X*, T4) (Fig. 5). Thus, an aqueous solution containing 1.0% CaC_2 and 0.5% AC or 0.5% CaC_2 and 0.5% AC will force pineapple and reduce the farmer's costs of forcing. Based on the results, we suggest that one application of 1.0% CaC_2 supplemented with 0.5% AC or two applications of 0.5% CaC_2 containing 0.5% AC at a 48 h interval would be sufficient to completely force reproductive development in pineapple. Because the sensitivity to forcing of pineapple cultivars in different environments is highly variable, these results may need to be adapted to the various cultivars and environments where pineapple is grown. We also tried to enhance the effect of ethephon solution with AC (0.5–1.0%), but no significant improvement was observed by this addition (data not shown). This may be because ethephon must be absorbed into plant tissue before ethylene is released from the ethephon molecule.

Conclusion

Pineapple shoot apical tissue releases more ethylene than does leaf tissue after cold stress. Hence, this tissue is most suited for further study of low-temperature induced ethylene production, associated enzyme activities (ACC synthase and ACC oxidase) and their relationship with pineapple flowering. Four applications of ice or ice water at 24 h intervals induced flowering of 'Tainon 17' pineapples under field conditions and the efficacy, though delayed, was comparable to CaC_2 and ethephon treatments. While forcing of organically-produced pineapple with ethylene is permitted in some countries where the use of ethylene is banned, ice treatments could be used to force pineapple for these restrictive markets. Other experiments showed that two applications of CaC_2 or ethephon were sufficient to force reproductive development in 'Tainon 17' pineapple in the field, and the effectiveness of CaC_2 was enhanced by the addition of 0.5% AC. The combination of CaC_2 and AC would reduce the cost of forcing because a reduced amount of chemical is required and only one application appears to be needed. Our findings have direct relevance to pineapple cultivation and therefore, this knowledge can be extended to other cultivars of pineapple as well, however, with required modifications in the forcing schedule.

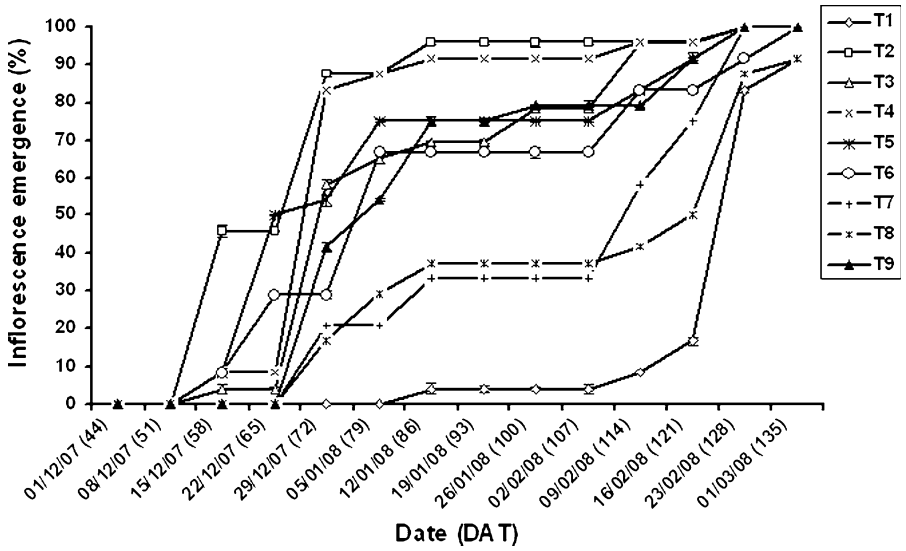

Fig. 5 Forced induction of flowering of 'Tainon 17' pineapple after treatment with an aqueous solution of CaC_2 with or without activated charcoal during the 2007–2008 season. The treatments were T1, water control (25°C); T2, 1.0% CaC_2 (2*X*); T3, 1.0% CaC_2 (1*X*); T4, 1.0% CaC_2 + 0.5% AC (1*X*); T5, 1.0% CaC_2 + 1.0% AC (1*X*); T6, 1.0% CaC_2 + 2.0% AC (1*X*); T7, 0.5% CaC_2 (2*X*); T8, 0.5% CaC_2 (1*X*); T9, 0.5% CaC_2 + 0.5% AC (1*X*). '*X*' denotes the indicated number of applications at a 48 h interval. Each data point represents the mean ± SE of three replicates ($n = 24$)

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References

Bartholomew DP, Malezieux E, Sanewski GM, Sinclair E (2003) Inflorescence, and fruit development and yield. In: Bartholomew DP, Paull R, Rohrbach KG (eds) The pineapple: botany, production and uses. CABI Publishing, Wallingford, UK, pp 167–202

Coppens d'Eeckenbrugge G, Leal F (2003) Morphology, anatomy and taxonomy. In: Bartholomew DP, Paull R, Rohrbach KG (eds) The pineapple: botany, production and uses. CABI Publishing, Wallingford, UK, pp 13–32

da Cunha GAP, Costa JTA, Reinhardt DH (2003) Natural flowering in pineapple: inhibition by growth regulators. Fruits 58:27–37

Devadas VS (2005) Standardisation of leaf sampling for nutrient analysis in pineapple var. Mauritius. IV International Pineapple Symposium. Acta Hortic 666:191–192

Friend DJC (1981) Effect of night temperature on flowering and fruit size in pineapple (*Ananas comosus* (L.) Merr.). Bot Gaz 142:188–190

Friend DJC, Lydon J (1979) Effects of daylength on flowering, growth, and CAM (Crassulacean acid metabolism) of pineapple (Ananas comosus (L.) Merrill). Bot Gaz 140:280–283

Gowing DP (1961) Experiments on the photoperiodic response in pineapple. Am J Bot 48:16–21

Hepton A (2003) Cultural system. In: Bartholomew DP, Paull R, Rohrbach KG (eds) The pineapple: botany, production and uses. CABI Publishing, Wallingford, UK, pp 109–142

Huang JY, Lin CH (2003) Cold water treatment promotes ethylene production and dwarfing in tomato seedlings. Plant Physiol Biochem 41:283–288

Hyodo H, Tanaka K, Watanab K (1983) Wound-Induced ethylene production and 1-Aminocyclopropane-1-carboxylic acid synthase in mesocarp tissue of winter squash fruit. Plant Cell Physiol 24:963–969

Kende H (1993) Ethylene biosynthesis. Annu Rev Plant Physiol Plant Mol Biol 44:283–307

Kuan CS, Yu CW, Lin ML, Hsu HT, Bartholomew DP, Lin CH (2005) Foliar application of aviglycine reduces natural flowering in pineapple. HortScience 40:123–126

Lin CH, Maruthasalam S, Shiu LY, Lien WC, Loganathan M, Yu CW, Hung SH, Ko Y, Chen YY (2009) VI International Pineapple Symposium. Acta Hortic 822:117–124

Py C, Lacoeuilhe JJ, Teisson C (1984) L'ananas: sa culture ses produits. Maisonneuve GP and Larose, Paris

Py C, Lacoeuilhe JJ, Teisson C (1987) The pineapple, cultivation and uses. Maisonneuve GP. Paris, pp 568

Soler A, Teisson C, Dole B, Marie Alphonsine PA (2006) Forcing in pineapple: what is new?. In: Bartholomew DP (ed) Pineapple News (ISHS) 13:27–31. http://www.ishs-horticulture.org/workinggroups/pineapple/PineNews13.pdf

Van de Poel B, De Proft JCMP (2009) Determination of pineapple (*Ananas comosus*, MD-2 hybrid cultivar) plant maturity, the efficiency of flowering induction agents and the use of activated carbon. Sci Hortic 120:58–63

van Overbeek J, Cruzado HJ (1948a) Note on flower formation in the pineapple induced by low night temperatures. Plant Physiol 23:282–285

van Overbeek J, Cruzado HJ (1948b) Flower formation in the pineapple plant by geotropic stimulation. Am J Bot 35:410–412

Wang RH, Hsu YM, Bartholomew DP, Maruthasalam S, Lin CH (2007) Delaying Natural flowering in pineapple through foliar application of aviglycine, an inhibitor of ethylene biosynthesis. Hortscience 42:1188–1191