Off-season Flower Induction of Longan with Potassium Chlorate, Sodium Chlorite, and Sodium Hypochlorite

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SUMMARY. Flower induction of longan (*Dimocarpus longan*) with potassium chlorate has improved the availability of longan fruit, but potassium chlorate is potentially explosive and often difficult to purchase, transport, and store. Previous reports suggested that hypochlorite enhances natural longan flower induction. This study is the first to demonstrate that chlorite- and hypochlorite- (bleach) induced off-season longan flowering is similar to chlorate-treated trees. Hypochlorite induction of flowering with bleach was likely the result of chlorate in the bleach solution. Chlorate was present in the leachate from potted longan trees treated with bleach and was detected in bleach before soil application. The quantity of chlorate found in bleach induced flowering to the same or greater extent as equivalent quantities of potassium chlorate, suggesting chlorate is an a.i. responsible for longan flowering.

Longan is in the Sapindaceae family along with its other better known relatives, lychee (*Litchi chinensis*) and rambutan (*Nephelium lappaceum*). Commonly referred to as dragon eye, the longan fruit is smooth, round, and yellowish brown at harvest with a translucent and crispy edible aril surrounding a round, brown-black seed. Although usually consumed fresh, the dried longan fruit is used for medicinal purposes or as a tea during special occasions (Zee et al., 1998).

Longan trees are commonly grown in many subtropical and tropical countries with the majority of the production in Thailand, Taiwan, China, and Australia (Menzel et al., 2002). In the United States, major

production occurs in Florida, Hawaii, and California. In 1998, the United States produced 1.4 million lb of longan with an estimated value of \$2.8 million (Mossler and Nesheim, 2002). In Hawaii, production was valued at \$438,000 for 142,000 lb of longan sold in 2005. Future longan production is expected to increase with current fruit production based on only $\approx 47\%$ of the total acreage planted in longan trees [U.S. Department of Agriculture (USDA), National Agricultural Statistics Service and Hawaii Department of Agriculture, 2006].

Floral initiation in longan is dependent on cool and dry environmental conditions with flowering occurring during March to May in Hawaii (Sabhadrabandhu

and Yapwattanaphun, 2001; Zee et al., 1998). Inconsistent floral induction and alternate bearing in longan production has been alleviated by the discovery of potassium chlorate (KClO_3) to induce off-season flowers and fruits worldwide (Choo, 2000; Manochai et al., 2005; Nagao and Ho-a, 2000; Sabhadrabandhu and Yapwattanaphun, 2001; Yen et al., 2001). Application of KClO_3 as a soil drench (10% to 20% chlorate solution), broadcast under the canopy (200 to 400 g/tree), foliar spray (1 to 2 g·L⁻¹), or branch or stem injection (0.05 to 0.25 g·cm⁻¹ branch) is effective for promoting flowering (Manochai et al., 2005; Yen, 2000; Yen et al., 2001). For optimal flowering, KClO_3 should be applied to trees with mature leaf flushes ≈ 40 to 45 d old (Manochai et al., 2005). Although KClO_3 induces flowering regardless of the genotype, the flowering response of each cultivar may vary with 100% flowering observed in 'Si-Chompoo' after application of 1.0 g·m⁻², whereas 'E-Daw' required 8.0 g·m⁻² (Manochai et al., 2005). Fruits obtained from KClO_3 -treated plants do not contain chlorate, chlorite, or hypochlorite residues in the aril, and the size, weight, exocarp color, fruit pole strength, fruit volume, total soluble sugars, and sulfur dioxide residues from postharvest treatment are unaffected (Kanaree and Pankasemsuk, 2005a, 2005b).

Originally discovered as the a.i. in fireworks gunpowder, KClO_3 is a strong oxidizing agent responsible for the explosive nature of fireworks (Yen, 2000; Yen et al., 2001). Potassium chlorate is an extremely useful

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
318.3268	fl oz/ft ²	mL·m ⁻²	0.0031
0.3048	ft	m	3.2808
0.0929	ft ²	m ²	10.7639
3.7854	gal	L	0.2642
2.5400	inch(es)	cm	0.3937
0.4536	lb	kg	2.2046
1	micron	μm	1
28.3495	oz	g	0.0353
305.1517	oz/ft ²	g·m ⁻²	0.0033
7.4892	oz/gal	g·L ⁻¹	0.1335
11.1612	oz/inch	g·cm ⁻¹	0.0896
1	ppb	μg·L ⁻¹	1
1	ppm	mg·L ⁻¹	1
14.7868	tablespoon	cm ³	0.0676
(°F - 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

tool to effectively plan flowering and fruiting of longan trees for market. However, the dangers associated with large quantities of this chemical make it difficult to obtain and store; a mixture of KClO_3 and sulfur was responsible for an explosion at a longan processing plant in Chiang Mai, Thailand, killing 35 workers and injuring over 100. Lack of personal protection equipment during prolonged use of KClO_3 by Thai longan workers resulted in increased levels of anemia, thrombocytopenia, high serum creatinine, and methemoglobinemia, which are hypothesized to be related to KClO_3 toxicity (Wiwatanadate et al., 2001).

Sodium hypochlorite (NaOCl) is the a.i. in bleach and has been shown to increase flowering of 'Do' longan trees (53.7% flower emergence in untreated trees versus 96.2% flower emergence in treated trees) (Sritontip et al., 2005a). However, it is not clear if NaOCl can induce off-season flowering because soil drenches with NaOCl at $50 \text{ mL} \cdot \text{m}^{-2}$ of canopy produced only 5% flower emergence compared with KClO_3 $5 \text{ g} \cdot \text{m}^{-2}$ canopy, which produced 75% of flower emergence (Sritontip et al., 2005b). The objective of this experiment was to determine if NaOCl can be used as an alternative to potassium chlorate for off-season longan flower induction in Hawaii.

Materials and methods

EXPT. 1. Four-year-old 'Egami' and 'Biew Kiew' longan trees on the University of Hawaii, Waiakae Agriculture Research Station (WRS), were used for this study. The soil consisted of an extremely stony Papai muck with organic soils formed over mostly fragmented a'a lava. On 15 Sept. 2004, the longan trees were untreated or treated with 300 g/tree KClO_3 or 300 g/tree NaClO_2 applied as a granular broadcast under the tree canopy or 2 gal/tree liquid NaOCl bleach solution [397 mL (397 g) NaOCl a.i.] or 2 gal/tree bleach plus 0.5 g copper chloride (CuCl_2) applied as a soil drench under the tree canopy. The experiment was a completely randomized design with three trees per treatment. Two 'Egami' trees and one 'Biew Kiew' tree were randomly assigned to each treatment. On 10 Dec. 2004, flowering stopped and percent flowering

was calculated by recording the number of flowering terminals divided by the total number of terminals in the tree canopy.

EXPT. 2. The experiment was conducted in a well-drained silty clay loam soil in Onomea, Hawaii, where mean maximum and minimum temperatures were 26 and 19°C , respectively, and annual rainfall was $\approx 3781 \text{ mm}$. The experiment was repeated twice (18 May 2005 and 16 Sept. 2005). Three-year-old 'Biew Kiew' trees were selected for uniformity in vegetative flushing, and each treatment was randomly assigned to three trees (May application) or five trees (September application). Plants were treated as follows: no treatment control; 300 g/tree (May) or 250 g/tree KClO_3 (September) broadcasted under the canopy; 45 g/tree (May) or 50 g/tree KClO_3 (September) broadcasted under the canopy; and 2 gal bleach (May and September) applied as a soil drench under the tree canopy. After the KClO_3 applications, 2 gal of water was applied on the soil surface. Flowering was monitored biweekly by counting the number of flowering and nonflowering terminals on two sides (north and south) of the tree and determining the percentage of flowering terminals.

STATISTICAL ANALYSIS. SAS (SAS Institute, Cary, N.C.) PROC GLIMMIX was used for a generalized linear models approach with a logit link for the binomial responses (Littell et al., 2002). Treatment means were tested for a decrease from the KClO_3 standard using Dunnett's adjustment for multiplicity. In Expt. 2, test of effects slices were used to compare the north and south tree faces within treatments.

CHLORATE ANALYSIS. Two-year-old 'Biew Kiew' plants were grown at the USDA, Agricultural Research Service, Pacific Basin Agricultural Research Center, Tropical Plant Genetic Resource Management Unit at WRS in 15-gal pots containing a 1:1:1 (by volume) mixture of soil, composted macadamia husks, and volcanic cinder. Plants were kept in a covered greenhouse and fertilized bimonthly with 1 tablespoon of 16N-7P-13.3K plus micronutrients and monthly with 1 tablespoon/L of 30N-4.4P-8.4K foliar fertilizer. Plants were watered three times weekly with $\approx 19 \text{ L}$ water.

On 10 Aug. 2005, three potted trees were irrigated with either 1) 0.5 L water, 2) 0.5 L bleach (26.25 g NaOCl), or 3) 26.25 g KClO_3 granular broadcast with 0.5 L water. Plants were irrigated to maintain adequate soil moisture for growth but avoiding excess water leaching through the pot. One month after application, the pots were uniformly irrigated with $\approx 10 \text{ L}$ of water. Gravitation water from the soil was collected and volume recorded. The water samples were filtered with a $0.2\text{-}\mu\text{m}$ filter to remove soil particles and sent to NovaChem Laboratories Inc. (Oxford, Ohio) and analyzed for chlorate and chlorite according to the National Environmental Laboratory Accreditation Conference standard using ion chromatography (Environmental Protection Agency Method 300.1). Commercial bleach (5.25% NaOCl) samples were diluted 1:10 with distilled, deionized water and also analyzed for chlorate (NovaChem Laboratories).

SOIL ANALYSIS. Soil samples from three 'Biew Kiew' trees treated with 2 gal bleach and three untreated trees grown at the Onomea site were analyzed for salinity and sodium (Na^+) content. Fourteen weeks and 1 year after treatment, four 16-cm core samples were taken 50 cm from the trunk. Subsamples from each tree were pooled and sent to the University of Hawaii, College of Tropical Agriculture and Human Resources, Agricultural Diagnostic Service Center, Honolulu.

Results and discussion

EXPT. 1. Flowering of trees at the WRS treated with KClO_3 , NaClO_2 , bleach, and bleach plus CuCl_2 began 5 weeks (22 Oct. 2004) after treatment. By 12 weeks after treatment, trees stopped production of panicles and the number of flowering terminals was determined. Nontreated control trees did not flower and KClO_3 , NaClO_2 , bleach, and bleach plus CuCl_2 -treated trees exhibited 97.8% (484 flowering/495 total terminals), 91.8% (462 flowering/503 total terminals), 84.7% (326 flowering/385 total terminals), and 96.9% (493 flowering/509 total terminals) flowering, respectively. The means for each treatment were not significantly less than the KClO_3 treatment

($P > 0.27$) for NaClO_2 , bleach ($P > 0.18$), and bleach plus CuCl_2 ($P > 0.43$) as analyzed by SAS PROC GLIMMIX using Dunnett's adjustment for multiplicity. This demonstrates that chlorate, chlorite, and hypochlorite can effectively induce off-season flowering of longan.

Investigation into the mechanism of chlorate toxicity by Åberg suggested that the chlorate toxicity is incited by reduction of chlorate to chlorite and hypochlorite by nitrate reductase (reviewed by LaBrie et al., 1991). As predicted by Åberg, the majority of plant nitrate reductase enzymes reduce chlorate to the toxic chlorite. Chlorate was used extensively in the past as an herbicide to control problematic weeds such as bindweed (Latshaw and Zahnley, 1927; Loomis et al., 1933; Neller, 1930) and is the a.i. in many herbicides used today (Bennett and Shaw, 2000). Chlorate has also been useful in the isolation of mutants with reduced nitrate uptake or impaired nitrate reductase activity (Crawford and Forde, 2002; Crawford and Glass, 1998; LaBrie et al., 1991; Meyer and Stitt, 2001). Nitrate reductase activity in leaves is reduced in longan trees treated with soil applications of KClO_3 , NaClO_2 , and bleach (Matsumoto et al., in press).

Reduced nitrogen levels in foliage is hypothesized to be an important factor in determining the receptiveness of the terminals to natural flower induction conditions in lychee, a related species in the family Sapindaceae. Nitrogen levels in excess of 1.85% results in vegetative flushing, whereas levels of 1.75% to 1.85% are conducive to flowering (Menzel et al., 1988). Optimal lychee flowering and fruit set have been correlated with 1.3% to 1.5% nitrogen in leaf dry matter in Hawaii, South Africa, India, and Australia (Davenport and Stern, 2005), thus suggesting a possible link between low nitrogen levels and flowering in these Sapindaceae crops.

Hypochlorite enhances flowering in longan similar to potassium chlorate (Sritontip et al., 2005a), and we demonstrate that it can also effectively induce off-season flowering. NaOCl degradation occurs through two pathways. The first pathway leads to the production of oxygen, whereas the second leads to chlorate formation (Adam and

Gordon, 1999). The incorporation of copper (Cu^{2+}) to the bleach solution, an ion that catalyzes both degradation pathways of hypochlorite to chlorate or oxygen (Adam and Gordon, 1999), appeared to induce flowering to the same degree as KClO_3 treatment or bleach treatments alone, suggesting chlorate in bleach contributed to the promotion of longan flowering.

CHLORATE ANALYSIS. To apply equivalent quantities used in field applications of 300 g KClO_3 broadcasted in a 5-ft diameter (19.63 ft^2) under the tree canopy, 26.25 g KClO_3 and 500 mL (26.25 g of NaOCl) bleach were applied to potted longan plants in a 17-3/4-inch-diameter (1.72 ft^2) pot. Leachate collected 4 weeks after treatment contained $173.3 \pm 7.1 \text{ mg}\cdot\text{L}^{-1}$ of chlorate from pots treated with 26.25 g KClO_3 and $8.3 \pm 2.3 \text{ mg}\cdot\text{L}^{-1}$ of chlorate from pots treated with 0.5 L bleach. Leachate from control pots contained negligible chlorate (less than $50 \mu\text{g}\cdot\text{L}^{-1}$). Presence of chlorate in the leachate from the bleach treatments suggests that hypochlorite was converted to chlorate in the soil or before the application.

Decomposition of bleach to chlorate is also catalyzed by higher temperatures. Nonperishable products

such as bleach may be exposed to elevated temperatures during transportation and storage, which could account for the presence of chlorate in the bleach. Chemical analysis of the bleach resulted in $3.97 \pm 0.65 \text{ g}\cdot\text{L}^{-1}$ sodium chlorate, which is equivalent to 25 to 35 g of chlorate contained in the 2 gal of bleach applied to the trees.

EXPT. 2. To determine if the amount of chlorate found in bleach could induce longan flowering to the same extent as bleach, 'Biew Kiew' trees in Onomea, Hawaii, were left untreated or treated with 300 g KClO_3 , 45 g KClO_3 , or 2 gal of bleach. The 300-g treatment was previously shown to be effective for inducing flowering in Hawaii (Nagao and Ho-a, 2000), and 45 g of KClO_3 is a high estimate of the amount of chlorate present in 2 gal of bleach applied onto the soil.

Trees treated on 18 May 2005 began flowering 8 weeks after treatment (8 Aug. 2005) and produced new panicles until 16 weeks after treatment. No significant differences in flowering were found between the north and south tree faces for all treatments. Bleach-treated trees flowered at 54.9% (118 flowering/215 total), which is significantly less ($P = 0.048$) than the 300 g KClO_3 treatments in which 94.0% (221

Fig. 1. Percentage of 'Biew Kiew' longan trees with panicles after treatment with 2 gal (7.6 L) bleach, 50 g (1.8 oz) potassium chlorate (KClO_3), or 250 g (8.8 oz) KClO_3 on 16 Sept. 2005. Trees were monitored every 1 to 2 weeks from 3 Nov. 2005 to 6 Jan. 2006.

flowering/235 total) of the terminals flowered, but the bleach treatment was not significantly different ($P = 0.406$) compared with 67.9% (142 flowering/209 total) of the terminals flowering in trees treated with 45 g KClO_3 . This suggests that the chlorate in the bleach may be responsible for flower induction in longan.

Trees treated on 16 Sept. 2005 began flowering 7 weeks after treatments (4 Nov. 2005) and actively produced new panicles until 12 weeks (8 Dec. 2005) after treatment (Fig. 1). Flowering on the north and south faces of the trees was not significantly different for 250 g KClO_3 ($P = 0.926$) or 2 gal bleach ($P = 0.783$); however, flowering was significantly different for the 50 g KClO_3 treatment ($P = 0.003$). This difference in the flowering observed between the different faces of the trees treated with 50 g KClO_3 may be attributed to the uneven distribution of chlorate on the soil under the tree canopy. Overall flowering of the trees treated with bleach was 97.1% (297 flowering/306 total terminals), which was not significantly different ($P = 0.501$) than trees treated with 250 g KClO_3 in which 96.4% (318 flowering/330 total) of the terminals flowered but was significantly greater ($P = 0.004$) than flowering on trees treated with 50 g KClO_3 in which 68.7% (237 flowering/345 total) of the terminal flowered.

Comparing longan flowering observed in trees treated in May and September, KClO_3 at the higher (250 or 300 g) application rate consistently resulted in over 90% flowering and the lower (45 or 50 g) KClO_3 application rate resulted in $\approx 70\%$ flowering. Flowering of longan trees treated with bleach varied between the two applications dates with 54.9% flowering observed after the 18 May 2005 application and 97.1% flowering observed after the 16 Sept. 2005 application. This increase in flowering may be attributed to the conversion of NaOCl to chlorate possibly by heat, prolonged storage, or interaction with elements in the soil. We experimentally determined that 25 to 35 g of chlorate is present in 2 gal of bleach. However, if fully converted to chlorate, 2 gal of bleach would be equivalent to ≈ 397 g of chlorate, which would result in increased flowering.

In addition to hypochlorite and chlorate, sodium was also added to the soil during the bleach treatment. Soil analyses from trees treated with bleach and control trees at Onomea showed that at 14 weeks after treatment, soil from bleach-treated trees contained 151.3 ± 36.8 ppm of Na^+ , whereas soil from untreated control trees contained 37.7 ± 2.9 ppm of Na^+ . Soil pH and salinity were not significantly different in soil samples from bleach-treated and untreated trees and no visible signs of sodium toxicity could be detected. One year after bleach treatment, there was no significant difference between soil samples of bleach-treated trees, 24.4 ± 2.9 ppm, and untreated trees, 24.3 ± 4.8 ppm, suggesting that sodium did not accumulate in the soil.

Therefore, we conclude that chlorate, chlorite, and bleach are able to induce flowering in longan. Bleach is an effective alternative to potassium chlorate for producing off-season longan fruits. The decomposition of hypochlorite to chlorate in the bleach contributes to the floral induction without sodium accumulation in soil 1 year after application in a high rainfall environment.

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