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Emulsions of long-chain fatty acids as fruit sizing and ripening agents for gibberellin-A_3-treated rabbiteye blueberries (*Vaccinium virgatum* Aiton syn. *V. ashei*)

Blair J. Sampson*, Donna A. Marshall, Stephen J. Stringer, Hamidou F. Sakhanokho, Christopher T. Werle

United States Department of Agriculture-Agricultural Research Service, Thad Cochran Southern Horticultural Laboratory, 810 Hwy 26 West, Poplarville, MS 39470, USA

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ABSTRACT

Some rabbiteye blueberry cultivars (*Vaccinium virgatum* Aiton syn. *ashei*) lose ≤67% of their berries to a floral polymorphism (*z1-5*) that reduces male and female function by deforming corollas, stamens, and pistils. These deformities give flowers an asymmetric shape, and if severe enough, could cause sterility. Overcoming sterility and associated yield loss in 'Premier' flowers was possible with two applications of an exogenous plant growth regulator (PGR), 250 ppm gibberellin-A_3 (GA). Interestingly, the dual emulsifier and surfactant chosen for our GA dips, 0.5% coconut oil soap, behaved like a PGR by inducing adventitious embyony, possibly apomixis. Resulting berries were 20–25% heavier and matured 7–14 days earlier. Fruit sets as high as 80–90% stemmed from the emulsion's GA ingredient. Yet, the heaviest seed-rich berries either resulted from cross-pollinated flowers or from unpollinated ones treated with GA/coconut oil soap emulsion (hereafter known as the emulsion). A second greenhouse experiment incorporated chemical dips containing four concentrations of coconut oil soap, five component fatty acids with medium length to long-chain aliphatic tails (C_{10}–C_{18}), and three new cultivars. Again, these replicated trials achieved similar results. GA stimulated high fruit set, while coconut oil soap plus its component long-chained fatty acids produced heavier faster growing berries for unpollinated blooms of 'Austin', and 'Prince'. 'Tifblue' flowers however yielded seedless fruit, indicating genotypic sensitivities to exogenous GA.

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1. Introduction

Profitable blueberry farming throughout warm temperate biomes hinges on lucrative low-chill cultivars of rabbiteye blueberry and southern highbush blueberry. A major advantage of growing early-ripening low-chill blueberries is the synchronization of harvest with premium market prices. Unfortunately, cultivated blueberry production is often limited by a high degree of genetic self-incompatibility and by small delicate blossoms prone to physical injury, poor pollination, and sometimes a genetic disorder affecting viability (NeSmith and Krewer, 1999; Marshall et al., 2006). This recently identified disorder is a floral polymorphism of certain rabbiteye blueberry cultivars with 'Ethel' in their pedigree (e.g.,'Premier', 'Yadkin', and 'Columbus', Sampson et al., 2013). The trait profoundly deforms flowers thereby reducing viability, pollinator visitation, and berry yield. We call these polymorphic flowers *z1-5* zygomorphs because of an asymmetric shape and five distinct categories of structural deformity to corollas, stamens, and pistils (Fig. 1). The exact triggers affecting the expression of *z1-5* phenotype in rabbiteye blueberry *Vaccinium virgatum* Aiton are currently unknown. However, various homeotic genes or single mutations found in many other floral mutants may affect *z1-5* phenotype. Environmental factors such as temperature during bud set may also alter *z1-5* expression thereby increasing the frequency and the severity of floral deformities (Running, 1997; Ohto et al., 2009; Zhang et al., 2010). Extreme *z1-5* floral expression can render as much as two-thirds of a blueberry bush sterile (Sampson et al., 2013).

Blueberry flowers whether damaged or non-functional may still set adequate fruit provided ovaries remain intact and responsive to suitable exogenous plant growth regulators (PGRs). Exogenous

Abbreviations: LCFAs, long chain fatty acids; GA_3, gibberellin-A_3, gibberellic acid; PGR, plant growth regulator.

* Corresponding author. Tel.: +1 6014038765; fax: +1 6017954965.
E-mail addresses: blair.sampson@ars.usda.gov, Blairsampson@bellsouth.net (B.J. Sampson).

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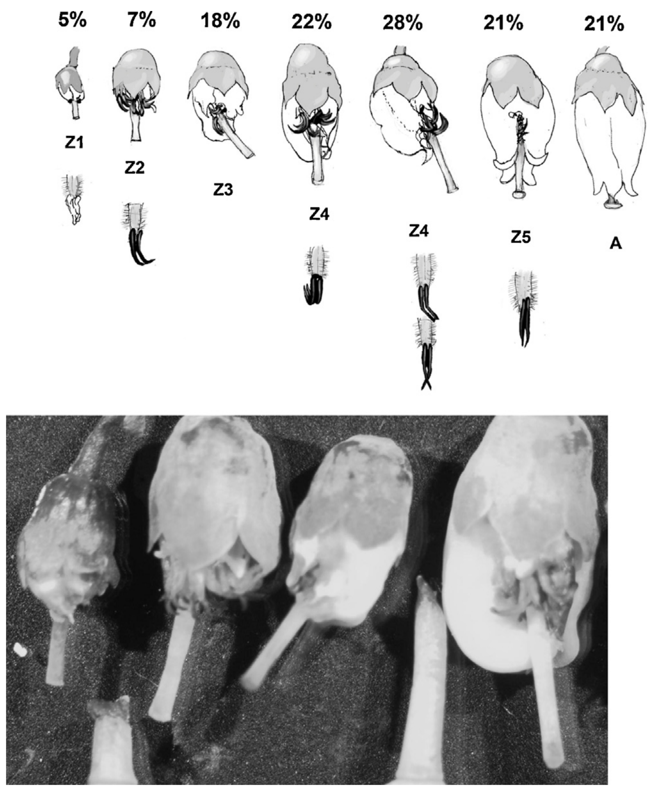

Fig. 1. Illustration (from Sampson et al., 2013) and photograph of five representative forms of polymorphic flowers (polymorphs = zygomorphs: z1-5) of 'Premier' rabbiteye blueberry showing various defects in corollas and anthers. The wild-type (a = actinomorph) is also provided for comparison. The frequency of each morph is shown as a percentage.

gibberellin-A_3 or gibberellic acid, abbreviated GA_3 or GA, induces parthenocarpic fruit set in warm-temperate blueberry species like rabbiteye blueberry. Gibberellins are a diverse class of tetracyclic diterpenoids containing 100+ chemical species. Many are merely biosynthetic precursors, whereas others are bioactive at low concentrations, have specific target sites, and endogenously regulate multiple metabolic pathways. At the whole plant level, these pathways are critical to bud differentiation, vegetative growth, anthesis, flower sex determination, pollen tube growth, fruit set, endosperm growth, ovule development, and seed germination (Vendrell, 1970; Dweikat and Lyrene, 1988; Pharis and King, 1985; Singh et al., 2002; Ohto et al., 2009; Chen et al., 2012). An upside of spraying low doses of 250–500 ppm GA_3 emulsion onto intact blueberry ovaries is an incredible burst of fruit set (Ogilvie et al., 1991; NeSmith and Krewer, 1999). The downside of course is the setting of seedless berries, which are half the size of pollinated berries, and being slow to mature, sell for less money (Cano-Medrano and Darnell, 1998).

GA could also play a role in setting seed in fruit crops like blueberry. We know exogenous GA bolsters blueberry fruit set, perhaps through the suppression of fruit abscission. However, blueberry ovules rarely develop into seeds despite gibberellins' regulatory role in embryogenesis. In sundry other crops, GA can chemically induce the setting of both fruits set and seeds. GA elevates levels of apomixis or asexual seed set if only slightly ~1% for specific genotypes of corn and roses (Dubois and deVries, 1986; Hu et al., 1991; Ogilvie et al., 1991). Both pollen function and ovule growth also depend on gibberellins, especially in horseradish, where GA drives pollen tube growth and regulates gene expression during embryogenesis (Singh et al., 2002; Ohto et al., 2009; Chen et al., 2012). GA also stimulates a greater production of fruit and seed in prunes through the induction of earlier bud break (Niederholzer and Glozer, 2004). In a related stone fruit crop, peach, direct injection of GA into young ovules promotes seed set by preventing embryos from aborting (Stutte and Gage, 1990). Given its role as a potent regulator of early seed development, perhaps exogenous gibberellin can be made to induce seed set in blueberry (Ben-Cheikh et al., 1997).

In 2010, fruit commodity groups requested a remedy for poor fruit set in z1-5 rabbiteye blueberries. We chose GA_3, a PGR applied before ovule fertilization to induce high levels of parthenocarpy. Our GA emulsion included de-ionized water, technical grade GA_3, and coconut oil soap as a surfactant. A pilot study was conducted in 2010 with a single cultivar, Premier. A second study in 2011 tested biological activity of GA and coconut oil based on increases in fruit set, berry size, maturation, sugar content, and seed set for three field-grown cultivars of rabbiteye blueberry. The emulsion's synergistic effect on *V. virgatum* fruiting responses and GA's hormonal action minus its soap emulsifier were assessed by comparing two GA mixtures, one with GA plus coconut oil, and the other with GA plus Silwet, a presumably inert non-ionic surfactant. A third follow-up study in 2013 was an expanded greenhouse experiment using three different cultivars first to identify biologically active fatty acids constituting the coconut oil emulsifier, second, to determine the ideal concentration of the coconut oil soap eliciting peak positive fruiting responses, and third, to compare berry quality for cross-pollinated blooms and unpollinated flowers treated with GA emulsion. Overall goals of the three studies were to:

(1) Determine if wildtype and z1-5 type blueberry flowers respond to GA treatment.
(2) Test the potency of GA emulsions to produce larger, faster-developing seeded berries that approach the quality of fruit resulting from cross-pollination.
(3) Examine genotypic responses to the GA emulsion; i.e., assess the reliability of GA emulsion to produce marketable quality fruit for select cultivars of rabbiteye blueberry.

2. Materials and methods

2.1. Experiment 1

GA's ability to boost berry set for z1-5 flowers was tested using 32 two-year-old potted 'Premier' plants moved into a 10 m × 15 m greenhouse bay at the USDA-ARS Southern Horticultural Laboratory in Poplarville, Mississippi, USA (latitude 30.85N, longitude 89.46W). Although potted plants were well watered by automated drip and fertilized with Osmocote, they still produced 93% *z1-5* flowers. Therefore, we haphazardly chose 100 terminal inflorescences, each bearing ~25 open blooms and randomly assigned one of five treatments: manual cross-pollination, two doses of 250 ppm technical grade GA emulsion (Sigma–Aldrich, St. Louis, MO), two control solutions, and a combination of manual pollination and GA dips. Bias among flower counts was normalized across each treatment by having each observer ($n = 3$) tag one-third of terminals. The first treatment (*SOAP* or *emul*) tested the soap's (brand: Ready-For-Use hand soap, Carroll Co., Garland, TX) PGR activity and controlled for GA's net effect on blueberry fruit set and growth. Our soap emulsifier contained 10% coconut oil, citric acid, and a non-cellulosic gelling agent and was mixed in deionized water at 0.5% (v/v). The second treatment was a control to confirm the soap surfactant's PGR activity and to account for natural rates of parthenocarpy in test plants; it included 20 unpollinated terminal inflorescences dipped into de-ionized water (*UP*). The third treatment [$GA_3 + UP(emul)$] tested the GA emulsion's and its soap emulsifier's ability to enhance fruit set, seed set, and berry growth rate for unpollinated flowers. Ingredients of the emulsion included 250 ppm technical grade GA_3 (Sigma–Aldrich,