Fruit Set of Rabbiteye Blueberry (*Vaccinium ashei*) after Subfreezing Temperatures

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ABSTRACT. Plants of 'Brightwell' and 'Tifblue' rabbiteye blueberry (*Vaccinium ashei* Reade) were subjected to 0, -1, -3, or -4.5 °C for 1 hour during flowering. After treatment, half of the plants were exposed to bees (*Bombus* sp.) only, and half were exposed to bees and received applications of GA₃. Fruit set of both 'Brightwell' and 'Tifblue' pollinated by bees declined sharply after exposure to -1 °C for 1 hour, but there was no visible damage to corollas, styles, and ovaries. Fruit set of GA₃-treated plants of both cultivars equaled that of control plants (plants having no cold exposure) at temperatures ≥ -3 °C. Both pollinated and GA₃-treated plants had ≤ 2% fruit set after exposure of flowers to -4.5 °C. Both prefreeze and postfreeze applications of GA₃ were beneficial for fruit set. Assessment of flower part damage at the different temperatures indicated corollas were most sensitive to freeze damage, followed by styles, and then ovaries. Results suggest fertilization and fruit set of pollinated rabbiteye blueberries can be greatly impaired by even mild freezes (-1 to -2 °C), whereas, appropriately timed applications of GA₃ can result in little reduction in fruit set even after moderate freezes (-3 to -4 °C) of blueberries during bloom. Chemical name used: gibberellic acid (GA₃).

Freeze damage is a peril faced by blueberry (*Vaccinium* sp. L.) growers throughout the southeastern United States and other growing regions, with some fruit loss occurring almost every year. Unlike other fruit crops such as peaches [*Prunus persica* (L.) Batsch.], where freeze damage can actually aid in thinning the crop, even mild freeze damage to blueberries often results in significant yield reductions. Eck (1988) suggested that 60% to 80% of blueberry flowers must set fruit for attainment of commercial yields. Thus, a means of reducing crop losses due to freeze damage would have immediate economic impact.

Large commercial fields of rabbiteye blueberries (*Vaccinium ashei*) often exhibit poor fruit set under natural pollination conditions (Lyrene and Crocker, 1983; Lyrene and Goldy, 1983). Fruit set of several rabbiteye blueberry cultivars can be enhanced by application of GA₃ when there is insufficient pollination (NeSmith and Krewer, 1997b). Activity of the growth regulator depends on both rate and stage of flower bud development (NeSmith and Krewer, 1992; NeSmith and Krewer, 1997a). Hence, progress has been made in using GA₃ effectively as a management tool to improve rabbiteye blueberry fruit set under poor pollination conditions (Williamson et al., 1995), and the growth regulator has been used successfully by growers over the past few years in the Southeast.

Stimulation in the growth of freeze-injured peach fruit by GA₃ has been reported (Zucconi and Bukovac, 1978). Also, Modlibowska (1975) found that gibberellins applied after decapitation of styles increased yield of 'Bramley' apples [*Malus sylvestris* (L.) Mill. var.

domestica Borkh. Mansf.] through induction of parthenocarpic fruit set. Recent research has indicated that inducing fruit set of rabbiteye blueberry after freeze damage to flowers is possible with applications of GA₃ (NeSmith et al., 1995). However, only one subfreezing temperature (-2.5 °C) was used in this study. The lower temperature limit at which GA₃ remains active in inducing blueberry fruit set needs to be determined.

Cold injury to rabbiteye blueberry flower buds differs depending on the stage of development (Spiers, 1978). Temperatures ≤ -1 °C caused considerable injury to flowers with expanded, opened corollas (Spiers, 1978). Silva (1995) reported there were differences in susceptibility of flower parts of rabbiteye blueberries to subfreezing temperatures. Although these investigations documented injury to flowers, they did not assess postfreeze fruit set. Gupton (1983) purported that rabbiteye blueberry flower injury following subfreezing temperatures, although not always visible, may prevent successful fertilization and fruit set. Additional research is needed to determine the degree of blueberry fruit set resulting from natural (i.e., bee) pollination following a range of subfreezing temperatures. Therefore, the objectives of this research were to examine the influence of GA₃ and bee pollination on fruit set of rabbiteye blueberries exposed to various subfreezing temperatures, and to assess injury to various flower parts after cold exposure.

Materials and Methods

One-year-old 'Tifblue' and 'Brightwell' plants grown in pots in pine bark were obtained from a commercial nursery during Fall 1995. At the Georgia Station plants were subjected to 700 h of artificial chilling (<7 °C) in a dark coldroom. After chilling, blueberries were moved to a greenhouse with average day/night temperatures of 24/18 °C and a natural photoperiod to force budbreak. As flowers began to open, a small hive of commercial bumble bees (*Bombus* sp.) containing ≈50 workers was placed in the greenhouse. Bee activity on the flowers was excellent. When most flowers were at stage 5 to 6 of development (Spiers, 1978), GA₃ and freezing treatments were imposed.

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Freezing cycles were accomplished overnight in controlled-environment growth chambers. Cooling rates were set to simulate conditions in the field (2 to 5 °C drop in temperature per hour). Fig. 1 illustrates the freezing cycles from the four growth chambers used in the experiment. Plants of each treatment were randomly assigned to growth chambers. The minimum temperatures achieved in the chambers (± 0.2 °C) were 0, -1, -3, and -4.5 °C, respectively. A group of plants also remained in the greenhouse (≥ 18 °C) to serve as a control. Temperatures in the plant canopy during the time in growth chambers were recorded using a LI-1000 data logger (LI-COR, Lincoln, Nebr.) and a shielded thermistor. After the cycle of sub-freezing temperature exposure, blueberries were returned to the greenhouse with bees for 4 weeks and were randomly assigned to benches. Additional nontreated flowering plants of 'Tifblue', 'Brightwell', and 'Climax' were placed in the greenhouse to insure an adequate supply of pollen. Half of the plants of each cultivar were randomly selected from each temperature regime to receive a GA₃ (ProGibb 4%, Abbott Laboratories, Chicago, Ill.) application ≈ 4 h after returning to the greenhouse. A second application of the growth regulator was made 14 d later. The remaining half of the plants received no GA₃. All GA₃ applications consisted of spraying with a solution of 250 mg·L⁻¹ GA₃ with 0.25% X-77 surfactant. Plants were sprayed to the point of drip with the solution. There were six plants of each cultivar for each temperature and GA₃ treatment combination.

The day after temperature treatments, flower buds were counted on each plant and were classified according to stage of development (Spiers, 1978). The average number of flowers per cluster was determined for each cultivar by counting flowers for 100 flower clusters. These average values (6.5 flowers per cluster for 'Brightwell', 6.6 flowers per cluster for 'Tifblue') were used to estimate total numbers of flowers per plant from flower cluster counts. Fruit set was assessed 12 weeks after treatment by counting the number of fruit on the plants and dividing by the estimated total number of flowers per plant. Data analysis consisted of mean and standard error calculations.

A second experiment was conducted with only 'Tifblue' to examine the effect of prefreeze and postfreeze applications of GA₃ on fruit set. Experimental procedures were the same as described previously, with the following exceptions. No bees were used in the second experiment; hence, the control plants were not pollinated. Also, only a single application of GA₃ was made either before or after the subfreezing temperature cycles. The prefreeze application of GA₃ was made 16 h before plants were placed in the growth chambers. The postfreeze GA₃ application was made 4 h after plants were removed from chambers. Gibberellic acid applications and fruit set assessment were as described previously. Six plants were randomly assigned to each treatment and data were analyzed by calculating means and standard errors.

A separate, small-scale freezing test was conducted on flowers of 'Brightwell' to assess flower part sensitivity to subfreezing temperatures utilizing methods described by Lindstrom and Dirr (1989). Briefly, detached flowers of the cultivar at stage 5 and 6 of development (opened or nearly opened blooms) were subjected to a controlled freezing bath. Flower clusters were wrapped in cheese cloth and were placed in glass tubes. The tubes were placed in a freezing bath at 2 °C for 1 h, and then they were nucleated with ice. Samples remained at 2 °C for another 2 h and then were cooled at a rate of 2 °C·h⁻¹. Flowers were removed at predetermined intervals of 0, -2.5, -3.0, -3.5, -4.0, or -4.5 °C. Flower clusters were allowed to thaw for 4 h under

refrigeration (4 °C), and then flower part damage was rated. At least 30 blooms were exposed to each temperature. Damage ratings were made for corollas, styles, and ovaries. To rate damage, oxidative browning was observed on the flower parts, and the number of damaged flowers was recorded. No attempt was made to separate degree of damage into slight, moderate, or severe. However, most damage noted was moderate to severe. Anther damage was difficult to assess, so this flower part was not included. Percentages of flower parts damaged were calculated, and these data were used to generate freeze damage curves for the various flower parts. The individual flowers at each temperature were treated as a population, hence, no variation measure was possible.

Results

The different temperature regimes resulted in highly variable damage to flowers of the whole plants used in the first two experiments (data not shown). There was no visible damage to flower buds at any stage at 0 or -1 °C. At -3 °C, moderate damage was observed, and at -4.5 °C severe damage occurred for flowers at stages 4, 5, 6, and 7 of development. In general, flower buds at lesser stages of development had little or no damage at -3 °C.

'Tifblue' fruit set in response to GA₃ and to bees only for the different temperature regimes is illustrated in Fig. 2. With bees only, fruit set of 'Tifblue' reached a maximum of 25% to 30%. There was no difference in fruit set between plants in the control and the 0 °C temperature regime for the bees only treatment. However, at -1 °C fruit set from natural pollination diminished to $\leq 20\%$. At -3 °C, fruit set for the bees only treatment was $< 5\%$. In contrast, 'Tifblue' plants treated with two applications of GA₃ displayed fruit set of 50% to 60% at -1 °C. At -3 °C fruit set of the GA₃-treated plants had only declined to $\approx 50\%$. Plants in both natural pollination and GA₃ treatments set $< 2\%$ of their fruit at -4.5 °C.

Results for 'Brightwell' were somewhat similar to those for 'Tifblue', but 'Brightwell' fruit set from natural pollination overall was generally greater (Fig. 2). As with 'Tifblue', 'Brightwell' fruit

Fig. 1. Growth chamber temperature cycles for exposure of rabbiteye blueberries to different temperature regimes during flowering.

Fig. 2. Influence of gibberellic acid (GA_3) and bees on fruit set of 'Brightwell' and 'Tifblue' rabbiteye blueberries subjected to different subfreezing temperature regimes. Freezes were imposed when most flowers were at stage 5 to 6 of development (opened and nearly opened blooms). Vertical bars represent SE with $n = 6$.

set with only bees declined to 20% after -1°C , while GA_3 -treated plants exhibited fruit set in excess of 60% at the same temperature. At -3°C , 'Brightwell' plants exposed only to bees had $\leq 10\%$ fruit set, whereas those plants receiving two growth regulator applications set $\geq 50\%$ of their fruit. Again, at -4.5°C both bee-pollinated and GA_3 -treated plants set $\leq 2\%$ of their fruit.

Results from the experiment examining prefreeze and postfreeze applications of GA_3 to 'Tifblue' indicated the two application strategies resulted in similar fruit set (Fig. 3). Fruit set of the control in this experiment was extremely low due to the absence of bees and pollination. There was a tendency for greater fruit set with the postfreeze application of GA_3 at -3°C . As in the previous experiment, fruit set approached 0% for 'Tifblue' when plants were exposed to -4.5°C , regardless of GA_3 application method.

Exploration of flower part damage due to subfreezing temperatures for 'Brightwell' re-

vealed that the flower parts differed in their sensitivity to temperature (Fig. 4). Corollas were the most sensitive to subfreezing temperatures (as determined by oxidative browning), followed by styles and then ovaries. There was no visible damage to any of the flower parts at $\geq -2^\circ\text{C}$. At -4.5°C , 100% of all parts of 'Brightwell' stage 5 and 6 flowers showed some degree of freeze damage.

Discussion

Low fruit set values for control plants of 'Tifblue' resulting from pollination are similar to fruit set values reported for this cultivar under field conditions (NeSmith and Krewer, 1997a). Also, Lyrene and Goldy (1983) reported that many rabbiteye blueberry cultivars exhibit naturally low fruit set. Of the 11 cultivars they observed, 'Tifblue' had the poorest fruit set. Fruit set for pollinated 'Brightwell' not subjected to freezing temperatures in the current study was similar to those values reported for several other cultivars by Lyrene and Goldy (1983).

The fact that GA_3 increased fruit set of both 'Brightwell' and 'Tifblue' control plants as compared to pollination alone is similar to previous findings (NeSmith and Krewer, 1997a; NeSmith et al., 1995). Additionally, Cano-Medrano and Darnell (1998) concluded that although GA_3 applications had no beneficial or detrimental effects on fruit set of 'Beckyblue' rabbiteye blueberry under optimal pollination conditions (pollen saturation by hand pollination), fruit set was substantially increased under suboptimal pollination conditions by the growth regulator. The authors reported that $\geq 40\%$ of fruit resulting from partial pollination abscised by 42 d after anthesis. Hence, lack of a high degree of fruit set for naturally pollinated rabbiteye blueberries in a field environment likely results from incomplete pollination, even under above-freezing temperatures, and properly timed GA_3 applications can improve fruit set under these conditions.

Silva (1995) found that brief exposure of 'Tifblue' flowers at stage 6 of development to 0 and -5°C caused little oxidative browning of the corollas and ovules; however, 'Climax' and 'Pre-

Fig. 3. Influence of gibberellic acid (GA_3) on fruit set of 'Tifblue' rabbiteye blueberry when applied 16 h before or 4 h after exposure of plants to different subfreezing temperature regimes. Freezes were imposed when most flowers were at stage 5 to 6 of development (opened and nearly opened blooms). Vertical bars represent SE with $n = 6$.

Fig. 4. Damage sustained by corollas, styles, and ovaries of 'Brightwell' rabbiteye blueberry flowers subjected to different subfreezing temperature regimes. Freezes were imposed when most flowers were at stage 5 to 6 of development (opened and nearly opened blooms). Symbols represent the percentage of a population of at least 30 blooms at each temperature.

mier' showed some browning of corollas at these temperatures. The author reported further that oxidative browning was severe for anthers of 'Climax' and 'Premier' at 0 and -5 °C, but was only slight for anthers of 'Tifblue'. In contrast, Spiers (1978) indicated that 'Tifblue' flowers with a mean stage of development of 5.7 were severely damaged by 4 h at -1 °C. The temperature regimes of the current study were designed to simulate natural freeze conditions caused by radiation cooling in the field. Therefore, the duration of temperature exposure in the current study and the observations reported on flower damage were essentially intermediate to those observations of Silva (1995) and Spiers (1978) for 'Tifblue'. This may explain why we observed less damage to flowers than Spiers (1978) at -1 °C, but considerably more damage than Silva (1995) at -4.5 °C.

Unlike Silva (1995) and Spiers (1978), the current investigation examined not only flower damage of rabbiteye blueberries, but also fruit set following subfreezing temperature exposure. Our results strongly suggest that the potential of fruit of rabbiteye blueberries to set by bee pollination declines readily after subfreezing temperature exposure of the flowers. Fruit set resulting from only pollination declined as the temperature to which flowers were exposed fell below -1 °C for 1 h. This finding is somewhat surprising because no damage to flower parts could be observed under these conditions. There is apparently hidden damage to flowers after only 1 h of exposure to -1 to -2 °C that impairs fruit set by pollination. Gupton (1983) reported that, depending on the rabbiteye blueberry cultivar, up to 67% of flowers with no visible damage failed to set fruit, even with hand pollination, after cold exposure. He speculated that pistils of the flowers had been damaged, even though not visibly, thus rendering successful fertilization improbable. The decline in fruit set observed in our investigation after exposure to -1 °C was not likely caused by anther damage after freezing as reported by Silva (1995), because an abundant supply of pollen was available from flowers that were never exposed to subfreezing temperatures. Therefore, one

cannot simply visually inspect flowers to assess potential loss due to freezing.

Results herein confirm an earlier report that suggested GA₃ could be used to set fruit on freeze-damaged rabbiteye blueberries (NeSmith et al., 1995). The lower temperature limit for a beneficial response appears to be around -4 to -3 °C, particularly for flowers at stages 5 to 6 (opened or nearly opened blooms). Hence, compared to pollination alone, there is an apparent temperature window of 3 to 4 °C in which fruit set could be improved by applying GA₃ after a freeze during bloom. Flowers with slight corolla browning and style damage were induced to set fruit with applications of GA₃, whereas, fruit set by pollination alone was greatly reduced for such flowers. However, when ovaries were severely damaged by freezing conditions, GA₃ was of little value. To assess the potential for using GA₃ to improve fruit set after mild to moderate freeze situations, flowers should be examined closely to determine the extent of freeze damage to ovaries. It is essential that decisions to apply GA₃ be based on flower damage assessment

rather than reported air temperatures.

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