BEE FORAGING BEHAVIOR AND POLLINATING ACTIVITY ON RABBITEYE BLUEBERRY *VACCINIUM ASHEI* READE

by

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(Under direction of Keith S. Delaplane)

ABSTRACT

In this dissertation, based on research conducted in four years (2000 – 2003) at the University of Georgia, USA, are presented results of studies about bee foraging behavior on rabbiteye blueberry, an important commercial crop throughout the southeastern USA, interactions of honey bees (*Apis mellifera* L.) with carpenter bees (*Xylocopa virginica* (L.), and the use of honey bees as vectors of the biocontrol agent *Bacillus subtilis* (Ehrenberg) Cohn used to reduce the incidence of mummy berry disease.

These results demonstrate that pollination efficacy of honey bees on rabbiteye blueberry was pollinator density-dependent. The data indicate that *V. ashei* var. 'Climax' responds positively to increases in honey bee density as measured by fruit-set, seed number, and speed of ripening. Within a range of 400 – 6,400 bees there was a trend for a corresponding increase in fruit-set with means ranging from 25.0 to79 percent.

This study suggests that rabbiteye blueberry is an important energetic resource for pollinators. Nectar standing crop and nectar production during 24 h were affected by age of flowers and increased significantly in flowers older than one day. Nectar standing crop varied as

well over the blooming season. Average nectar volume per flower was 2.85 µl, with an average sucrose concentration of 29.45%.

Following a 2-yr study conducted to assess how nectar robbing in honey bees affects fruit production in rabbiteye blueberry, it was concluded that under tent conditions the continuous presence carpenter bees results in nearly 40% incidence of *A. mellifera* robbery. This level of secondary nectar thievery does not reduce fruit-set but does reduce the number of seeds per berry.

On average, honey bees spent more time and energy handling flowers during legitimate visits than during robbing, suggesting that an energetic advantage exists for honey bees that engage in secondary nectar thievery in *V. ashei*.

Further this study confirmed the ability of honey bees to vector the biocontrol agent *B. subtilis* to blueberry flowers and suggests that application of Serenade (commercial formulation of *B. subtilis*) via hive-mounted dispensers is a promising strategy to reduce the potential for transmission of mummy berry disease when honey bees are used as blueberry pollinators.

Overall this research increases our understanding of pollinating efficacy and behavioral interactions of bee visitors in rabbiteye blueberry.

INDEX WORDS: Honey bees, *Apis mellifera*, carpenter bees, *Xylocopa virginica*, pollination, rabbiteye blueberry, *Vaccinium ashei*, foraging, nectar production, nectar larceny, mummy berry, biocontrol, *Bacillus subtilis*

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DEDICATION

To my lovely wife Rajmonda and my wonderful boys Raseld and Bergin, whose unconditional support, encouragement and love helped me complete this dissertation.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Bees and plants have lived together for millions of years. During this coexistence they continuously have changed and tried to adapt to each other to benefit reciprocally. Humans whose number and needs have been growing constantly have tried to increase the productivity of plants and animals that constitute the basic source of food for them. In this process a better understanding and knowledge of living things (especially animals and plants), their interactions, and complex relationships become priorities, not only to increase production for human benefit, but to protect and sustain the world in which we live.

This dissertation is structured in seven chapters, five of which (2-6) present the main part of this research, and constitutes an attempt to better understand the relationship between bee foraging behaviors and pollinating activity on rabbiteye blueberry, the interaction of honey bees with other bees (especially carpenter bees), and to shed light on the possibility of using honey bees as vectors of a biocontrol agent to reduce the incidence of mummy berry disease in blueberry.

Blueberries are native to several regions in the United States including all the states along the Atlantic seaboard (Eck 1988). Blueberries are long-lived, extremely attractive bushes with no thorns making them an excellent addition to any farm or garden. Several wild species of blueberry have been improved through a selection and breeding program and now are grown

commercially in many parts of the United States and Canada, the largest producers (83 %) of blueberries in the world (USDA 2003a).

Blueberry. This vascular plant (subkingdom Tracheobionta) belongs to Spermatophyta subdivision (seed plants), Magnoliophyta division (flowering plants), class Magnoliopsida (dicotyledons), order Ericales, family Ericaceae (heath family) and genus *Vaccinium* L. (USDA 2003b). The scientific name of the genus *Vaccinium* L. was established by Linnaeus in 1783 from material collected by Peter Koln in eastern North America (Butkus and Pliszka 1993). Alough considerable confusion exists over the taxonomic classification of blueberry (Eck et al. 1990), most authorities consider it a part of Vaccinieae, a subfamily of the Ericaceae (Galleta 1975).

Early work of selection initiated at the end of the nineteenth century in response to increased demand and wholesale price of this fruit, leading to the creation and improvement of hybrids and cultivars that are currently grown in commercial scale not only in North America, but also in 15 other countries in Europe, South America, Africa and Australia (Eck et al. 1990, Austin 1994). Three general types of blueberry are commercially grown today: highbush, lowbush and rabbiteye.

Highbush blueberry (*Vaccinium corymbosum*). The highbush blueberry is a term applied to those North American species that range in height from 5-23 ft (1.5-7 m). The cultivated highbush blueberry has been developed primarily from two species, *Vaccinium corymbosum* L. and *Vaccinium australe* Small. This species is cultivated mainly in Michigan, New Jersey and North Carolina (Austin 1994) as well as in some other areas of the USA (Horton et al. 1989). Recently interest in highbush blueberry has been rapidly increasing in southern areas

of the USA because its high quality and early ripening fruit make it desirable for blueberry growers (NeSmith 1999a).

Lowbush blueberry (*Vaccinium angustifolium*). The term lowbush blueberry applies to those species with height less then 3 ft (1 m) (Eck et al. 1990) and includes several species, among them *V. myrtilloides* Mischaux, which ranges from Vancouver to Maine, Quebec, and the maritime provinces of Canada (Austin 1994) and south to New York, Pennsylvania and Indiana. *V. angustifolium* Aiton is the most commercially important lowbush blueberry and the most abundant species in Canada (Barker et al. 1964).

Rabbiteye blueberry (*Vaccinium ashei*). Rabbiteye blueberry applies to those species with intermediate height (between highbush and lowbush) of 5-19 ft (1.5-6 m) (Eck et al. 1990). They are the most commercially important species of blueberries grown in southeastern USA. Rabbiteye blueberries are native to northern Florida, southern and eastern Georgia and southeastern of Alabama (Horton et al. 1989, Austin 1984, Eck 1988). In general the rabbiteye is the most adaptable, productive, vigorous and pest tolerant of the three types of blueberry mentioned here. Rabbiteye blueberries are generally adapted better to regions around 32˚ latitude both north and south of equator, and they can grow well in higher altitudes. The research on selection and hybridization conducted for more than three decades on this blueberry in Florida, Georgia and North Carolina has contributed to the most significant improvement in fruit quality of cultivated blueberry (Eck 1988). Due to these qualities and successful adaptation to many soils, the most commonly grown blueberry species in the Southeast is rabbiteye. The blueberry industry in Georgia and other southeastern states has expanded considerably during the past decade (Krewer and NeSmith 2002, Scherm and Krewer 2003). In Georgia more than 4400 acres (Anonymous 2003) are currently planted and this area is expected to increase in the future

(NeSmith 1999a). Georgia ranks 7th in blueberry production in the United States and is exceeded only by Maine, Michigan, New Jersey, North Carolina, Oregon and Washington. Georgia blueberry production in 2001 was $23 million (GAR 2003). In Georgia, blueberries are grown commercially primarily in the southern part of the state, but some are grown in the north Georgia mountains. The largest producers are Clinch, Bacon and Appling Counties (GAR 2003).

Among rabbiteye cultivars, Climax, Austin, Brightwell and Powder Blue are the best suited for mechanical harvesting for the fresh market. Climax blooms early and is more likely to suffer from spring freeze damage. Other cultivars which can be considered for commercial shipping in some situations are Premier (good producer, but marginally soft for mechanical harvest), Beckyblue (blooms very early), and Briteblue (mid-late season, slow growing), and Tifblue (cracks in some years) (Krewer and NeSmith 2002). New cultivars like Ochlockonee (late-ripening ability, high yield and medium-to-large berries) and Alapaha have been released in recent years by the University of Georgia (NeSmith 2004).

Health benefits. Blueberries are widely recognized as a healthful food (Sanchez-Moreno et al. 2003). Researchers at the USDA Human Nutrition Center (HNRCA) have found that blueberries rank number one in antioxidant activity when compared to 40 other fresh fruits and vegetables (Prior et al. 1998). They contain anthocyanins, the red/blue pigments that have substantial antioxidant activity (McGhie et al. 2003). Other studies have reported that blueberries also contain an array of phenolics, including quercetin, kaempferol, myricetin, chlorogenic acid and procyanidins that contribute to antioxidant capacity (Kalt et al. 1999, Sellapans et al. 2002). Blueberries have received much attention since being reported to contain high oxygen radical-absorbing capacity (ORAC) (Wang et al. 1996). Blueberries have been shown to have anti-aging

effects (Joseph et al. 1999), ability to reduce build up of so-called "bad" cholesterol and to reduce risk of urinary tract infection (Howell et al. 1998).

Blueberries are a good source of nectar for nectarivores. *Vaccinium* spp. attract a diversity of nectarivores. Thirty-four species from five bee families have been reported to visit lowbush blueberry, *V. angustifolium* (Finnamore and Neary 1978, Morrisette et al. 1985), while 27 species (including representatives of six of the seven bee families occurring in eastern North America) have been reported to visit rabbiteye blueberry (Cane and Payne 1993). Another congener, deerberry *V. stamineum* L., is visited by many species of seven bee families (Cane et al. 1985). Honey bee workers visit highbush blueberry to collect both nectar and pollen, but nectar foragers predominate (Dogterom and Winston 1999); thus it seems that floral nectar is the most important reward for honey bees.

Nectar is an important attractant for pollinators, and a plant's reproductive success can be influenced by the amount and concentration of nectar produced by flowers (Wesselingh and Arnold 2000). Nectar distribution affects pollinator movements and frequency of foraging bouts, whereas the number of visited flowers affects pollen dispersal and reproductive success of the plant (Real and Rathcke 1991, Kerans and Inouye 1993). Therefore measurements of nectar volume and quality are important in understanding pollinator energetics, nutrient requirements, and behavior in floral patches (Kerans and Inouye 1993, Williams 1997).

Pollination requirements and pollinators. Insect pollination is beneficial for blueberry production, especially for rabbiteye cultivars which are generally self-incompatible and require cross-pollination with another rabbiteye cultivar (Delaplane and Mayer 2000). Rabbiteye varieties are largely self-sterile (except variety 'Centurion') and require cross pollination with another suitable rabbiteye variety (El-Agamy et al. 1982). Rabbiteye blueberries often have low

fruit-set and unacceptable commercial yields (Lyrene and Crocker 1983), problems usually associated with poor pollination (Filmer and Marucci 1963). Cross-pollination with other varieties improves fruit-set, size, and earliness of ripening (Gupton and Spiers 1994).

The blueberry flower is well-suited for pollinators, especially buzz-pollinators which are able to vibrate the flower rapidly to release pollen from the pores of anthers. Blueberry pollen is sticky and relatively heavy. It is not easily blown by wind like pine or corn pollen. Further the shape and position of blueberry flower parts effectively prevent pollen from falling onto a receptive stigma, even in cultivars that are self-fertile. Therefore, in order to set fruit the flowers of a blueberry plant must be pollinated by insects. Buzzing or sonicating greatly increases the amount of pollen released by the flower, but even non-buzz pollinating species such as honey bees, can pollinate blueberry when bees are present in sufficient numbers and visit flowers legitimately.

Honey bees (*Apis mellifera* L.) are the most numerous bee visitors of blooming rabbiteye blueberries in south Georgia, USA, followed in descending order of abundance by bumble bee queens (*Bombus* spp.), bumble bee workers, carpenter bees, and southeastern blueberry bees (*Habropoda laboriosa* (F.)) (Delaplane 1995). Based on single-bee flower visits, *H. laboriosa* and *Bombus* queens were determined to be the most efficient pollinators of rabbiteye variety 'Tifblue,' and honey bees the least efficient (Cane and Pane 1990). This disparity is due in part to the non-specialized foraging habits of honey bees, their inability to sonicate (Delaplane and Mayer 2000), and the comparative inaccessibility of the 'Tifblue' flower to short-tongued honey bees. Despite these disadvantages, honey bees are widely recognized as a valuable resource for blueberry growers (Dorr and Martin 1966) to ensure adequate blossom pollination (Eck 1988). They are easily managed and available in large numbers all year long. Moreover, the success

with honey bees realized by Sampson and Cane (2000) with the rabbiteye variety 'Climax' suggests that the pollination efficacy of honey bees on *V. ashei* is variety-dependent.

Carpenter bees (*Xylocopa* Latreille) are common flower visitors in the Southeastern USA (Cane and Payne 1990, Delaplane 1995). Some flowers, including rabbiteye blueberry, possess long tubular corollae in which nectar is inaccessible to the carpenter bees, so the bees obtain nectar by imbibing it through perforations that they make with their maxillary galeae in the walls of the calyx or corolla. This behavior is called nectar theft or robbery (Faegri and Van der Pijl 1979, Inouye 1980, 1983). Carpenter bees have been reported to act as nectar thieves in other plants (Guitian et al. 1994, Scott et al. 1993), as well as blueberry (Cane and Payne 1990, Delaplane 1995, Sampson and Cane 2000). Nectar-thieving flower visits often do not result in pollination benefit to the plant (Inouye 1980, 1983, Maloof and Inouye 2000). The removal of floral nectar by robbers decreases the standing crop and in some cases changes the sugar concentration of nectar available to other pollinators (Pleasants 1983). Its effects on plant reproduction range from benign (Maloof 2001) to damaging (Inouye 1980, 1983, Maloof and Inouye 2000, Dedej and Delaplane 2004).

Moreover, the robbery holes in flowers made by nectar thieves *Xylocopa virginica* and *X. micans* are attractive to honey bees (*A. mellifera* L.) – a species commonly imported for commercial pollination. Honey bees are incapable of making robbery holes, but readily visit holes made by *Xylocopa* spp. and thus act as secondary nectar thieves (Delaplane and Mayer 2000). *Apis mellifera* learns to transition from aperture visits (legitimate) to illegitimate lateral visits (larceny) only after *X. virginica* becomes active in the orchard. Delaplane (1995) showed that 92.3% of honey bees were observed to rob nectar in the presence of *Xylocopa* spp.

Studies have shown that bees, including honey bees, can select and preferentially visit the most rewarding flowers in a patch (Morse 1980, Corbet et al. 1984) and avoid recently visited flowers (Giufra and Nunez 1992, Goulson et al. 2001, Stout and Goulson 2002). Other studies have documented the primary nectar thieving activity of carpenter bees and its secondary expression by honey bees, but no information was available before this study about the effect of these behaviors on the pollination efficacy of *A. mellifera* in rabbiteye blueberry.

It is important to understand not only the relationship and interaction between honey bees and carpenter bees in using the reward of blueberry flowers, but also to explain why honey bees prefer to visit perforated instead of intact flowers.

Honey bees, important pollinators of commercial blueberries, also vector diseases like mummy berry; therefore, strategies are needed to reduce the potential for disease transmission when honey bees are used as blueberry pollinators. Failure to produce good blueberry yields is often the result of freeze injury during bloom (NeSmith 1999b), poor pollination (Filmer and Marucci 1963, Lyrene 2004), and/or presence of disease (Scherm et al. 2001). Among the diseases affecting blueberry in Georgia, mummy berry, caused by the fungus *Monilinia vaccinii-corymbosi* Reade (Honey), has the greatest economic impact on the industry (Scherm et al. 2001).

Symptoms are manifested in the blighting of emerging leaves and shoots during spring and in the mummification of maturing fruit in summer due to infection of open flowers by conidia (asexual spores) transported to the floral stigmas by wind, rain, and especially pollinating insects (Batra and Batra, 1985, Cox and Scherm 2001, Woronin 1888) via the stigma-style pathway.

The involvement of pollinating insects in vectoring of *M. vaccinii-corymbosi* poses a dilemma for commercial blueberry producers. On one hand, enhancing pollinator activity, most commonly achieved by supplementing honey bees in the field (Scherm et al. 2001), is essential for ensuring effective pollination and adequate fruit set (Dedej and Delaplane 2003; Sampson and Cane, 2000). On the other hand, increased bee density is likely to lead to increased vectoring of the mummy berry pathogen. Thus, strategies are needed to reduce the potential for disease transmission when honey bees are used as blueberry pollinators.

Scherm et al. (2004) recently documented that the biofungicide Serenade, a commercial formulation of the bacterium *Bacillus subtilis* (Ehrenberg) Cohn, effectively controlled flower infection by *M. vaccinii-corymbosi* when applied directly to the stigmas of open flowers in the laboratory. Because honey bees can adsorb (Prier et al. 2001) and deliver formulated biocontrol agents in other pathosystems involving flower infection (Johnson and Stockwell 1998, Kovach et al. 2000, Maccagnani et al. 1999, Peng et al. 1992; Thomson et al. 1992, Yu and Sutton 1997), it is possible to mimic this type of dry-application using honey bees as vectors. Thus bee delivery of Serenade to open blueberry flowers in the field could reduce flower infection by *M. vaccinii-corymbosi* regardless of whether conidia of the pathogen are vectored by the bees themselves or by other means.

OBJECTIVES OF THE STUDY

The main objectives of the study were to evaluate (1) the importance of pollinators especially honey bees *Apis mellifera* L. on blueberry production, (2) the effects of varying densities of honey bees and (3) the interaction of honey bees with carpenter bees during flower visits and pollination. In studying the characteristics of nectar production on blueberry flowers

and behavior of bees visiting blueberry flowers, this research was intended not only to test the importance of blueberry flowers as a source of nectar for nectarivores, but also to understand and explain energetic involvement and effects of robbing behavior of honey bees on blueberry flower visitation and pollination. By studying the ability of honey bees to vector the biocontrol agent Serenade, it was intended to determine ways to reduce the risk of mummy berry disease on blueberry.

EXPECTED RESULTS

1 - Despite the fact that many bee species, native and exotic, are attracted to rabbiteye blueberry flowers, no objective descriptive information exists on the value of this plant as an energetic resource for nectarivores. As an important crop species native to the southeastern USA, I expected to make these characterizations in order to understand the relevance of *V. ashei* for initiatives in this region for pollinator conservation and sustainable agriculture.

2 - It is conceivable that an individually inefficient pollinator species, as such the honey bee (when considered based on measurements of single-bee flower visits), may perform satisfactorily if it is able to field a forager force large enough to increase the rate of legitimate flower visits. Based on this hypothesis I expected that increased honey bee density on rabbiteye blueberry flowers would result in higher rate of legitimate bee flower visits and consequently higher fruit set and better fruit quality.

3- Previous studies have documented the primary nectar thieving activity of carpenter bees and its secondary expression by honey bees, but no information is available about the effect of these behaviors on the pollination efficacy of *A. mellifera* in rabbiteye blueberry. Honey bees are effective pollinators of *V. ashei* var. ‘Climax’ but it is possible that their efficacy is

compromised when they are diverted to robbing behavior. I expected that the diversion to robbing behavior of honey bees would depend on the number of carpenter bees and it would affect the pollination efficacy of honey bees.

4 - Reducing the illegitimate behavior of honey bees induced by nectar thievery of carpenter bees would result in higher efficiency of honey bees as pollinators in rabbiteye blueberry. In my attempts to understand and explain this complex behavior I expected that the energy cost benefit would be the driving factor to explain why honey bees prefer to visit perforated instead of intact flowers on blueberry.

5 - Based on the ability of honey bees to adsorb bacterial spores, vector conidia to flowers (Batra and Batra 1985) and considering the demonstrated successful use of honey bees to disseminate beneficial bacteria and entomopathogenic viruses to different plants, I expected that honey bees would be able to deliver commercially formulated *B. subtilis* from hive-based dispensers to early age open blueberry flowers, thus suppressing incidence of fruit mummification caused by *Monilinia vaccinii-corymbosi*.

REFERENCES

Anonymous, 2003. Georgia annual crop summary, 2002. Georgia Farm Report 03, 2: 4.

Austin, M. 1994. Rabbiteye blueberries. Agscience, Inc. Auburndale, Florida.

Barker W. G., V. I. Hall, L. E. Alders, and G.W. Wood. 1964. The lowbush blueberry industry in eastern Canada. Econ. Bot.18: 357-365.

Batra, L.R., and S.W.T. Batra. 1985. Floral mimicry induced by mummy berry fungus exploits host's pollinators as vectors. Science 228: 1011-1013.

Butkus, V., and K. Pliszka. 1993. The highbush blueberry – a new cultivated apices. Acta Hort. 346: 81-86.

Cane, H.J., Eickwort, C. G., Wesley, R. F., and J. Spielholz. 1985. Pollination ecology of *Vaccinium stamineum* (Ericaceae: Vaccinioideae). Am. J. Bot. 72: 135-142.

Cane, J.H., and J.A. Payne. 1990. Native bee pollinates rabbiteye blueberry. Alabama Agric. Exp. Sta. 37(4): 4.

Cane, J.H., and J.A. Payne. 1993. Regional, annual and seasonal variation in pollinator guilds: intrinsic traits of bees (Hymenoptera: Apoidea) underlie their patterns of abundance at *Vaccinium ashei* (Ericaceae). Ann. Entomol. Soc. Am. 86: 577-588.

Corbet, S. A., C. J. C. Kerslake, D. Brown, and N. E. Morland. 1984. Can bees select nectar-rich flowers in a patch? J. Apic. Res. 23: 234–242.

Cox, K.D., and H. Scherm. 2001. Gradients of primary and secondary infection by *Monilinia vaccinii-corymbosi* from point sources of ascospores and conidia. Plant Dis. 85: 955-959.

Dedej, S., and K. S. Delaplane. 2003. Honey bee (Hymenoptera: Apidae) pollination of rabbiteye blueberry *Vaccinium ashei* var. 'Climax' is pollinator density-dependent. J. Econ. Entomol. 96: 1215-1220.

Dedej, S., and K. S. Delaplane. 2004. Nectar-robbing carpenter bees reduce seed-setting capability of honey bees (Hymenoptera: Apidae) in Rabbiteye Blueberry *Vaccinium ashei* Reade 'Climax.' Environ. Entomol. 33(1): 100-106.

Delaplane, K. S. 1995. Bee foragers and their pollen loads in South Georgia rabbiteye blueberry. Am. Bee J. 135: 825-826.

Delaplane, K.S., and D. F. Mayer. 2000. Crop pollination by bees. CABI Publishing, Oxon, UK, 344 pp.

Dogterom, M.H., and M. L. Winston. 1999. Pollen storage and foraging by honey bees (Hymenoptera: Apidae) in highbush blueberries (Ericaceae), cultivar bluecrop. Can. Entomol. 131: 757-768.

Dorr, J. E. and E. C. Martin. 1966. Pollination studies on the highbush blueberry, *Vaccinium corymbosum* L. Mich. Agric. Exp. Sta. Quart. Bull. 48: 437-448.

Eck, P. 1988. Blueberry science. Rutgers University Press, New Brunswick, NJ, USA.

Eck, P., R. E. Cough, I. V. Hall, and J. M. Spiers. 1990. Blueberry Management. *In* Small fruit crop management. Prentice-Hall, Inc. NJ, USA, pp. 273-333.

El-Agamy, S. Z. A.; W. B. Sherman, and P. M. Lyrene. 1982. Pollen incompatibility in blueberries (*Vaccinium* spp.) J. Palynol. 18: 103-12.

Faegri, K., and L. W. Van der Pijl. 1979. The principles of pollination ecology (third edition). Pergamon, Oxford, UK, 244 pp.

Finnamore, A.T., and M E. Neary. 1978. Blueberry pollinators of Nova Scotia, with a checklist of the blueberry pollinators in eastern Canada and north-eastern United States. Ann. Soc. Entomol., Quebec, 23: 168-181.

Filmer, R.S., and P.E. Marucci. 1963. The importance of honeybees in blueberry pollination. 31st Ann. Blueberry Open House Proc., North Carolina Agric. Exp. Sta., pp. 14-21.

Galleta, G. J. 1975. Blueberries and cranberries. In J. Janick and J.N. Moore (ed.). Advances in fruit breeding. Purdue University Press, West Lafayette, Ind, 623 pp.

GAR. 2003. Georgia agricultural resources, Blueberries. http://resources.caes.uga.edu/media/GAR/blueberries.htm.

Giufra, M., and J.A. Nunez. 1992. Honey bees mark with scent and reject recently visited flowers. Oecologia, 89: 113-117.

Goulson, D., J. W. Chapman, and W.O.H. Hughes. 2001. Discrimination of unrewarding flowers by bees: direct detection of rewards and use of repellent scent marks. J. Insect Behav. 14: 669-678.

Guitian, J., J.M. Sanchez and P. Guitian. 1994. Pollination ecology of *Petrocoptis grandiflora* Rothm. (Caryophyllaceae): a species endemic to the north-west part of the Iberian Peninsula. Bot. J. Linnaean Soc. 115(1): 19-27.

Gupton, C.L., and J.M. Spiers. 1994. Interspecific and intraspecific pollination effects in rabbiteye and southern highbush blueberry. HortScience. 29(4): 324-326.

Horton, D., P. Bertrand, and G. Krewer. 1989. Small fruit pest management and culture. Bulletin 1022. Cooperative Extension Service, University of Georgia, GA, USA.

Howell A. B., V. Vorsa, A. D. Marderosian, and L. Y. Foo. 1998. Inhibition of the adherence of p-fimbriated *Escherichia coli* to uroepithelial-cell surfaces by proanthocyanidin extracts from cranberries. N. Engl. J. Med. 339: 1085-1086.

Inouye, D.W. 1980. The terminology of floral larceny. Ecology 61: 1251-1253.

Inouye, D.W. 1983. The ecology of nectar robbing. *In* (B. Bentley and T. Elias, eds.) The biology of nectaries. Columbia University Press, New York, New York, USA, pp. 153-173.

Johnson, K.B., and V. O. Stockwell. 1998. Management of fire blight: A case study in microbial ecology. Ann. Rev. Phytopathol. 34: 227-248.

Joseph, J. A., B. Shukitt-Hale, N. A. Denisova, D. Bielinski, A. Martin, J. J. McEwen, and P. C. Bickford. 1999. Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. J. Neurosci. 19 (18): 8114-8121.

Kalt, W., J. E. McDonald, R. D. Ricker and Z. Lu. 1999. Anthocyanin content and profile within and among blueberry species. Can. J. Plant Sci. 79: 617-623.

Kearns, C.A., D. W. and Inouye. 1993. Techniques for the pollination biologist. University Press of Colorado, USA.

Kovach, J., R. Petzoldt, and G. Harman. 2000. Use of honey bees and bumble bees to disseminate *Trichoderma harzianum* 1295-22 to strawberries for *Botrytis* control. Biol. Control 18:235-242.

Krewer, G., and D. S. NeSmith. 2000. Blueberry cultivars for Georgia**.** University of Georgia Fruit Publication 00-2.

Krewer, G., and D. S. NeSmith. 2002. The Georgia blueberry industry: its history, present state, and potential for development in the next decade. Acta Hortic. 574: 101-106.

Lyrene, P. 2004. Early-ripening rabbiteye blueberries. Pp. 113-118 in: Krewer, G. (ed.). Proc. Southeast. Blueberry Conf., 9-11 Jan. 2004, Savannah, GA. Department of Horticulture, University of Georgia, Tifton.

Lyrene, P.M. and T.E. Crocker. 1983. Poor fruit set on rabbiteye blueberries after mild winter: possible causes and remedies. Proc. Florida State Hortic. Soc. 96: 195-197.

Maccagnani, B., M. Mocioni, M. L. Gullino, E. Ladurner. 1999. Application of *Trichoderma harzianum* by using *Apis mellifera* as a vector for the control of gray mould of strawberry: First results. IOBC Bull. 22:161-164.

Maloof, J.E. 2001. The effects of a bumble bee nectar robber on plant reproductive success and pollinator behavior. Am. J. Bot. 88: 1960-1965.

Maloof, J.E. and D.W. Inouye. 2000. Are nectar robbers cheaters or mutualists? Ecology 81: 2651-2661.

McGhie, T. K., G. D. Ainge, L. E. Barnett, J. M. Cooney, and D. J. Jensen. 2003. Anthocyanin glycosides from berry fruit are absorbed and excreted unmetabolized by both humans and rats. J. Agric. Food Chem. 51 (16): 4539-4548.

Morse, 1980. Behavioral mechanisms in ecology. Harvard University Press. 383 pp.

Morrisette, R., A. Francoeur, and J-M. Perron. 1985. Importance des abeilles sauvages (Apoidea) dans la pollinisation des bleuetiers nains (*Vaccinium* spp.) en Sagamie, Quebec. Rev. Entomol. Quebec, 30: 44-53.

NeSmith, D.S., 1999a. Blueberry research at the University of Georgia. 1999 research annual update. Research report 662.

NeSmith, D.S. 1999b. Effect of bee pollination and GA_3 on fruit size and maturity of three rabbiteye blueberry cultivars with similar fruit densities. HortScience. 34: 1106-1107.

NeSmith, D.S. 2004. Predicting fruit development period of rabbiteye blueberry cultivars using growing degree days. Small fruit news: 2 (2): 4-8.

Peng, G., J. C. Sutton, and P. G. Kevan. 1992. Effectiveness of honeybees for applying the biocontrol agent *Gliocladium roseum* to strawberry flowers to suppress *Botrytis cinerea*. Can. J. Plant Pathol. 14:117-129.

Prier K.R.S., B. Lighthart, and J. J. Bromenshenk. 2001. Adsorption model of aerosolized bacterial spores (*Bacillus subtilis* variety *niger*) onto free-flying honey bees (Hymenoptera: Apidae) and its validation. Environ. Entomol. 30 (6): 1188-1194.

Prior, R. L., G. H. Cao, A. Martin, E. Sofic, J. McEwen, C. O'Brien, N. Lischner, M. Ehlenfeldt, W. Kalt, G. Krewer, and C. M. Mainland. 1998. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. J. Agric. Food Chem. 46 (7): 2686-2693.

Pleasants, J.M. 1983. Nectar production in *Ipomopsis aggregata* (Polemoniaceae). Am. J. Bot. 70: 1468-1475.

Real, L.A., and R. J. Rathcke. 1991. Individual variation in nectar production and its effect on fitness in *Kalamia latifolia*. Ecology. 72:149-155.

Sampson, B. and J. Cane. 2000. Pollination efficiencies of three bee (Hymenoptera: Apoidea) species visiting rabbiteye blueberry. J. Econ. Entomol. 93: 1726-1731.

Sanchez-Moreno, C., G. H. Cao, B. X. Ou, and R. L. Prior. 2003. Anthocyanin and proanthocyanidin content in selected white and red wines. Oxygen radical absorbance capacity comparison with nontraditional wines obtained from highbush blueberry. J. Agric. Food Chem. 51 (17): 4889-4896.

Scherm, H., NeSmith, D. S., Horton, D. L., and Krewer, G. 2001. A survey of horticultural and pest management practices of the Georgia blueberry industry. Small Fruits Rev. 1(4): 17-28.

Scherm, H., and G. Krewer. 2003. Blueberry production in Georgia: Historical overview and recent trends. Small Fruits Rev. 2: 83-91.

Scherm, H., H. K. Ngugi, A. T. Savelle, and J. R. Edwards. 2004. Biological control of infection of blueberry flowers caused by *Monilinia vaccinii-corymbosi*. Biol. Control. 29: 199-206.

Scott, P.E., S.L. Buchmann, and M.K. Orourke. 1993. Evidence for mutualism between a flower piercing carpenter bee and ocotillo – use of pollen and nectar by nesting bees. Ecol. Entomol. 8(3): 234-240.

Sellappan, S., C. C. Akoh, and G. Krewer. 2002. Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. J. Agric. Food Chem. 50: 2432-2438.

Stout, J.C., and D. Goulson. 2002. The influence of nectar secretion rates on the responses of bumblebees (*Bombus* spp.) to previously visited flowers. Behav. Ecol. Sociobiol. 52 (3): 239-246.

Thomson, S., D. Hansen, and K. Flint. 1992. Dissemination of bacteria antagonistic to *Erwinia amylovora* by honeybees. Plant Dis. 76:1052-1056.

USDA. 2003a. Commodity highlight. Trends in the U.S. blueberry industry. Economic research service. Fruit and tree nuts outlook. FTS-305.

USDA. 2003b. Plant profile for Vaccinium. *Vaccinium* L., blueberry. http://plants.usda.gov/cgi_bin/plant_profile.cgi?symbol=VACCI

Wang, H.G., G. Cao, R. L. and Prior. 1996. Total antioxidant capacity of fruits. J. Agric. Food Chem. 44: 701-705 (1996).

Wesselingh, R.A., and M. L. Arnold. 2000. Nectar production in Louisiana iris hybrids. Int. J. Plant. Sci.161(2): 245-251.

Williams, S.C., 1997. Nectar secretion rates, standing crop and flower choice by bees on *Phacelia tanacetifolia*. J. Apic. Res. 36, 23-32.

Woronin, M., 1888. Über die Sclerotienkrankheit der Vaccinieen-Beeren. Mem. Acad. Imp. Sci. St.-Pétersbourg (VIIe Série) 36: 1-49.

Yu, H., and J.C. Sutton. 1997. Effectiveness of bumblebees and honeybees for delivering inoculum of *Gliocladium roseum* to raspberry flowers to control *Botrytis cinerea*. Biol. Control 10:113-122.

CHAPTER 2

NECTAR PRODUCTION DYNAMICS OF RABBITEYE BLUEBERRY *VACCINIUM ASHEI* READE AND ITS VALUE AS AN ENERGETIC RESOURCE FOR INSECT NECTARIVORES[1]

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ABSTRACT

A study was conducted on the value of cultivated *Vaccinium ashei* Reade as a floral resource for bees. Nectar standing crop varied over blooming season and with age of flower. Average volume of nectar standing crop per flower was 2.85 µl, with an average sucrose concentration of 29.45%. Nectar standing crop and nectar production during 24 h were affected by age of flowers and increased significantly in flowers older than one day. Sucrose concentration of nectar was inversely correlated with relative humidity at 24 h and 12 h preceding measurements as well as at the time of measurements.

KEY WORDS Apoidea / nectarivores / nectar production / flower age / climatic effects / Apidae

INTRODUCTION

Blueberry is an important commercial crop throughout North America, Europe, Australia, New Zealand, and Japan (Eck 1988). In the southeastern USA, most blueberries are one of three types: rabbiteye *Vaccinium ashei* Reade, northern highbush *V. corymbosum* L. and southern highbush, a hybrid of northern highbush and one or more southern *Vaccinium* species (Lang and Danka 1991, NeSmith 1999). In Georgia, more than 1821 ha of blueberry are under production (Anonymous 2003).

Vaccinium spp., especially blueberries, attract a diversity of nectarivores. Thirty-four species from five bee families have been reported to visit lowbush blueberry, *V. angustifolium* Ait (Finnamore and Neary 1978, Morrisette et al. 1985), while 27 species including representatives of six of the seven bee families occurring in eastern North America have been reported to visit rabbiteye blueberry (Cane and Payne 1993). Another congener, deerberry, *V.*

stamineum L., is visited by many species of seven bee families (Cane et al. 1985). Honey bee workers visit highbush blueberry to collect both nectar and pollen, but nectar foragers predominate (Dogterom and Winston 1999); thus, it seems that floral nectar is the most important reward for honey bees.

Measurements of nectar volume and quality are important in understanding pollinator energetics, nutrient requirements, and behavior in floral patches (Kerans and Inouye 1993, Williams 1997). Nectar distribution affects pollinator movements and frequency of foraging bouts, whereas the number of visited flowers affects pollen dispersal and reproductive success of the plant (Real and Rathcke 1991, Kerans and Inouye 1993). It has been shown that flowers providing high energetic rewards are more likely to receive a visit than a conspecific flower providing lower reward (Thomson 1988, Real and Rathcke 1991). Nectar is an important attractant for pollinators, and a plant's reproductive success can be influenced by the amount and concentration of nectar produced by flowers (Wesselingh and Arnold 2000). Insect nectarivores generally prefer nectars of comparatively high sugar concentrations because of their higher energy content. Sugars such as sucrose, glucose and fructose are the most abundant components of nectar (Corbet 2003).

Despite the fact that many bee species, native and exotic, are attracted to rabbiteye blueberry flowers, no objective descriptive information exists on the value of this plant as an energetic resource for nectarivores. As a crop species native to the southeastern USA, it is especially important to make these characterizations in order to understand the relevance of *V. ashei* for initiatives in this region for pollinator conservation and sustainable agriculture. Therefore, we studied patterns of nectar production and nectar sucrose concentration over flower

life and blooming season and effects of weather on nectar characters of rabbiteye blueberry *V. ashei*.

MATERIALS AND METHODS

General. The study was conducted during spring 2003 at a mature 15-y old orchard interplanted with rabbiteye varieties 'Climax' and 'Premier' at the Horticulture Farm of the University of Georgia, Oconee County, GA, USA. Anthesis (the opening of flowers) started after mid-March and continued up to mid-April, reaching 50% by the beginning of April.

Nectar was extracted without excising flowers using calibrated 1-5 µl disposable pipettes (Fisher Scientific, Pittsburgh, PA, USA). In some cases we tilted flowers to more easily collect nectar (Corbet 2003). Nectar volume was measured to the nearest 0.1 µl. Sucrose concentration was measured as % Brix (g sucrose per 100 g solution) (Corbet 2003) using a hand-held refractometer model MT-098 (Three-In-One Ent. Co., LTD., Taipei, Taiwan) with range of 0-80 % Brix and accuracy to the nearest 1%. The Brix percentage was temperature-corrected using the correction table provided by the manufacturer. The quantity of sucrose per sample (mg) was calculated by multiplying volume of nectar x temperature-corrected Brix x sucrose density values after Dafni (1992, p. 148).

Effect of Flower Age on 24-h Nectar Production. Production here is defined as the apparent secretion rate – the rate change of nectar solute content in undisturbed, unvisited flowers (Corbet 2003). Four 'Climax' rabbiteye plants were excluded from visitation by bees and all but the smallest nectarivores with two cages comprised of 1.8 x 1.8 x 1.8 m frames covered with Lumite screen (Bioquip, CA, USA). With the aim to measure daily nectar production in flowers of different age, 10 unopened flowers (stage five, after Spiers 1978) were individually

tagged. Subsequently at 24-h increments (between 12:00–14:00), nectar was removed and measured (µl) and % Brix measured in these flowers up to drop of the corollae, which occurred at day 4. Day 1 was defined as the day in which the flower opened. This protocol was performed twice (23-26 March and 26-29 March).

Effects of Time and Flower Age on Nectar Standing Crop. Nectar standing crop is an estimate of average nectar resources available (per unit area or per flower) at a given time (Dafni 1992, Corbet 2003). In this section we determined standing crop per flower (mg sucrose and volume (µl)). A total of 530 flowers ('Climax' and 'Premier') were sampled over 17 days (for this and the following two sections). Due to carpenter bee (*Xylocopa virginica* L.) nectar-thieving activity, many flowers were perforated with robbery holes but only intact flowers were used for nectar measurements. The effect of time (Julian days during sampling interval) was used as a fixed effect in statistical analyses (see below). Additionally, flowers were classified as 1-d old, 2-d old, and > 2-d old, and age class used as a fixed effect in statistical analyses. We are able to discriminate visually these age classes of blueberry flowers (Fig. 2.1).

Effects of Weather on Nectar Standing Crop. To investigate effects of weather on volume and concentration of nectar, data for ambient temperature (ºC), relative humidity (% RH), wind speed (m/s), and solar radiation (watt/m^2) were collected from a weather station near the orchard for every 15 minutes for each of the 24-h and 12-h periods preceding nectar measurements, as well as within 30 min of nectar measurements, and used for regression analyses (see below).

Statistical Analyses. The effects of flower age on 24-h nectar production as well as effects of flower age and time (Julian day) on nectar standing crop were analyzed with analysis of variance (ANOVA) recognizing flower age or Julian day as fixed effects and random error as

test term. Means were separated by Duncan's test, and differences accepted at the $\alpha \leq 0.05$ level (SAS Institute 1992). The relationship of nectar standing crop and sucrose concentration with ambient temperature, relative humidity, wind speed, and solar radiation within 24 h, 12 h and at time of measurements were analyzed with regression models testing for linear, quadratic and cubic effects (SAS Institute 1992).

RESULTS

Effect of Flower Age on 24-h Nectar Production. Age of flower (1-4 days) affected quantity of sucrose per sample (mg) (F=3.4; df=3,73; P=0.023) and percentage Brix (F=13.1; df=3,73; P=0.0001), but not nectar volume (µl) (F=2.3; df=3,73; P=0.0891). The quantity of available sucrose (mg) is higher in flowers at days 3-4 of anthesis (respectively 37% and 32% of total sucrose production) than in flowers at day 1 (7.8%) (Table 2.1). Correspondingly, the trend was the same for percent Brix, with higher values at days 2-4 (27.3 – 28.4%) than for day 1 (16.5%). Changes of quantity of sucrose (mg) and percent Brix were not associated with changes in nectar volume.

Effects of Time and Flower Age on Nectar Standing Crop. Time (Julian day) affected quantity of sucrose (F=10.82; df=17,421; P=0.0001), percentage of sucrose (F=21.78; df=17,421; P=0.0001) and nectar volume (F=17.10; df=17,512; P=0.0001). The quantity of sucrose, the most reliable indicator of energy produced by a flower, averaged 1.095 mg per flower (n=439) with a range of 0.05 – 4.56. Percentage of sucrose in nectar averaged 29.45 % (n=439) with a range of 7.74 – 66.08 %, while volume of nectar standing crop averaged 2.85 µl per flower (n=530) with a range 0 – 9.8. Our study showed variation in the quantity of sucrose as well as % Brix (Fig. 2.2) during the bloom period of rabbiteye blueberry.

Age of flower affected all variables measured for standing crop: quantity of sucrose *(F*=29.9; df=2,314; *P*=0.0001), sucrose concentration *(F*=29.7; df=2,314; *P*=0.0001), and nectar volume (*F*=30.4; df=2,393; *P*=0.0001). Quantity of sucrose (mg), sucrose concentration (% Brix), and nectar volume increased with aging of flowers (Table 2.2).

Effect of Weather on Nectar Standing Crop. We found no effects of air temperature, relative humidity, wind speed and solar radiation for 24 h ($P \geq 0.505$) and 12 h ($P \geq 0.332$) preceding measurements as well as at time of measurements ($P \geq 0.392$) on quantity of sucrose produced by rabbiteye blueberry flowers, but we did find effects of air temperature, relative humidity, wind speed and solar radiation on sucrose concentration.

Air temperature for 24 h preceding measurements varied with sucrose concentration in a quadratic relationship in which concentration decreased as temperature increased, then increased at higher temperatures ($r^2 = 0.52$, $P \leq 0.0063$) (Fig. 2.3a). The same relationship for 12 h preceding measurements was explained by a negative linear relationship ($r^2 = 0.45$, $P \leq 0.0031$) (Fig.2.4a). No significant relationship was verified between air temperature and sucrose concentration at time of measurement ($P \geq 0.128$).

Average relative humidity for 24 h ($r^2 = 0.48$, $P \leq 0.0022$) (Fig. 2.3b) and 12 h ($r^2 = 0.51$, $P \leq 0.0012$) (Fig. 2.4b) prior to measurements and at time of measurements ($r^2 = 0.35$, $P \leq 0.013$) (Fig. 2.5a) varied inversely with sucrose concentration of nectar.

Our results did not show any relationship of average wind speed for 24 h ($P \geq 0.225$) and 12 h ($P \geq 0.609$) preceding measurements with nectar concentration, but we found a positive relationship between wind speed and sucrose concentration at time of measurement ($r^2 = 0.34$, $P \leq 0.0147$) (Fig. 2.5b).

Solar radiation for 24 h ($r^2 = 0.31$, $P \leq 0.0195$) (Fig. 2.3c) preceding measurements and at time of measurements ($r^2 = 0.28$, $P \leq 0.0299$) (Fig. 2.5c) varied positively with sucrose concentration.

DISCUSSION

Effect of Flower Age on 24-h Nectar Production. We found that age of flower (1-4 days) affected quantity of sucrose per sample (mg) and percentage Brix, but not nectar volume (Table 2.1) and that changes of quantity of sucrose and percent Brix were not associated with changes in nectar volume. This supports the use of measures of solutes as an indicator of floral value, which is preferable to measurements of nectar volume (*contra* Gilbert et al. 1991, Lloyd et al. 2002). It is, in fact, the sucrose content of nectar that is the primary currency of interest in studies of energetic cost and benefit (Corbet 2003).

We found the highest quantity of sucrose produced on days 3-4 of anthesis (respectively 37% and 32% of total sucrose production) while Jablonski et al. (1985) found with highbush blueberry higher production at days 1-2 (respectively 44% and 32%). Temporal increases in floral reward can be explained as a strategy by plants to increase rates of pollinator visitation, especially under conditions of pollination deficit. Similarly, the stigmata of blueberry flowers continue to grow beyond the corolla apertures if the flowers experience poor rates of pollinator visitation (Austin 1994).

The trend of our data for percent Brix in rabbiteye blueberry fits with the results of former studies in highbush blueberry (Brewer and Dobson 1969, Jablonski et al. 1985). Moreover, our study suggests that the average sucrose concentration in rabbiteye blueberry

flowers is lower (16.5 – 28.4 %) compared with the average sucrose concentration in highbush blueberry of 33 – 46 % (Brewer and Dobson 1969, Jablonski et al. 1985).

Effects of Time and Flower Age on Nectar Standing Crop. In our study we were able to confirm temporal (Julian day) and floral age effects on nectar standing crop of rabbiteye blueberry flowers. Nectar standing crop increased significantly in flowers older than one day. The age of inflorescence has been shown to influence nectar secretion in lowbush blueberry in which both nectar volume and sucrose content increase throughout the bloom period (Wood 1961). Our study showed variation in the quantity of sucrose as well as % Brix (Fig. 2.2) during the bloom period of rabbiteye blueberry, but not in a pattern of linear chronological increase as found by Wood (1961). This suggests that inferences on these variables should be based on multiple measurements covering the whole bloom period. Nectar standing crop (quantity of nectar in a flower at a given time (Corbet 2003)) has been shown to vary over the course of a season (Kearns and Inouye 1993) and to be affected by temporal and spatial factors (Pleasants 1983, Kearns and Inouye 1993), age of flowers (Pyke 1978), feeding activity of nectarivores, resorption, microclimatic conditions, and plant species (Corbet 2003).

Effect of Weather on Nectar Standing Crop. We did not find effects of temperature, relative humidity, wind speed and solar radiation on quantity of sucrose produced by rabbiteye blueberry flowers, but we did find effects of these factors on nectar sucrose concentration (% Brix). Air temperature for the 24 h preceding measurements had a complicated quadratic relationship with sucrose concentration (Fig. 2.3a), but air temperature for 12 h preceding measurements varied negatively with sucrose concentration of nectar (Fig. 2.4a). Additionally, sucrose concentration varied inversely with relative humidity for 24 h (Fig. 2.3b) and 12 h (Fig. 2.4b) preceding measurements as well as at time of measurements (Fig. 2.5a). Increase of wind

speed (Fig. 2.5b) at time of measurements positively affected sucrose concentration of nectar. Increasing solar radiation for the 24 h preceding measurements (Fig. 2.3c) as well as at time of measurements (Fig. 2.5c) positively affected sucrose concentration of nectar.

Earlier studies have shown positive relationships between nectar production and solar radiation and air temperature, especially in herbaceous plants. These relationships have been generally less obvious in trees and shrubs (Shuel 1992). Percival (cited by Shuel 1992) found no relationship between daily nectar yields in raspberry and immediate climatic conditions. Mallick (2001) observed that nectar production in the flowers of Tasmanian leatherwood, *Eucryphia lucida* (Eucryphiaceae) was independent of temperature and humidity. Other studies have suggested that apparent secretion of nectar (Corbet 2003) is affected by solar radiation, temperature and water balance; however, these patterns are not universal (Kearns and Inouye 1993).

Solute concentration in nectar has been shown to change with time in response to changing ambient humidity (Corbet et al. 1979), generally inversely (Shuel 1992). Southwick et al. (1981) were able to explain variation of sucrose concentration in nectar for closed blossoms of *Philadelphus* sp. due to variation in relative humidity, and Bertsch (1983) reported a similar relationship for nectar concentration and volume in fireweed (*Epilobuim angistifolium*). Our results for *V. ashei* confirm the effects of climatic factors on sucrose concentration as confirmed for other plant species, but do not implicate climatic effects on quantity of sucrose produced. Apparently the quantity of sucrose produced is more responsive to other factors relating to plant physiology or orchard management.

Total Available Nectar Standing Crop per ha at Mid-anthesis, Total Seasonal Nectar Production per ha, and Implications for Pollinator Carrying Capacity. Considering

the measurements in this study and the number of rabbiteye blueberry plants per ha 1500 – 1800 (Horton et al., 1989), we estimate that 15-y old rabbiteye blueberry plants (1.5 – 1.8 m high) yield a total available nectar standing crop per ha at mid-anthesis equal to 28.89 – 34.67 L, which at 29.9 % sucrose represents 11.10 – 13.32 kg sucrose. The same bushes during blooming season produce a total of 130.75 – 156.91 L or 38.31 – 45.98 kg sucrose per ha. This estimate is close to the values of 33 – 44 kg sugar per ha found in highbush varieties by Jablonski et al. (1985).

These gross production estimates, combined with published bee energetic requirements, permit us to extrapolate carrying capacity of this agro-ecosystem for particular bee taxa. A normal honey bee (*Apis mellifera*) colony at the time of blooming of *V. ashei* has *ca*. 20,000 – 25,000 bees, of which half are foragers (Rogers and Gordon 1992) and of whom 58 – 60 % act as nectar foragers (Seeley 1985). A honey bee forager normally makes 7 – 13 trips per day (Gary 1992), carrying pollen loads of 10 – 30 mg and nectar loads ranging from 10 – 80 mg (Winston 1987, Gary, 1992). A bee collecting 30 mg nectar of 50 % sugar content will collect during one day *ca*. 3240 J, enough to pay for the cost of the trip and feed 10 nest mates (Harrison and Fewell 2002). Based on our measurement of total available nectar standing crop of flowers per ha (28.89 – 34.67 L with approximately 30 % sugar or 11.10 – 13.32 kg sugar), we estimate that one ha of rabbiteye blueberry is sufficient to feed 26 – 39 honey bee colonies per d at mid-anthesis.

While observing bumble bees (*Bombus impatiens*) visiting flowers of highbush blueberry from 9:30 –12:30, France et al. (2000) documented that one forager performs on average one trip per h with 30 flowers visited per trip. A bumble bee making 9 – 10 foraging trips per d (Heinrich 1979) would be able to visit *ca*. 300 flowers, collecting 855 µl nectar. Based on our calculations of standing crop, one ha of rabbiteye blueberry would support 113 – 162 bumble bee colonies (each with 250 – 300 bees). Heinrich (1979) reported that the net daily rate of sugar intake by a

colony of *B. vosnesenskii* amounts to 45 g. Considering this value, the available nectar at mid-anthesis in one ha rabbiteye blueberry is sufficient to support per day 245 – 296 colonies of this species.

Sampson and Cane (2000) suggest that a mature bush of *V. ashei* with *ca*. 10,000 flowers is able to sustain one nest of *Osmia ribifloris* Cockerell. Based on our measurements and considering a density of 1500 – 1800 plants per ha (Horton et al. 1989), at least 2020 – 2430 nests of this bee can be sustained in one ha of mature rabbiteye blueberry *V. ashei.*

This study suggests that rabbiteye blueberry, a native crop species in the southeastern USA, is an important energetic resource for pollinators and its culture a significant component of sustainable agriculture for this region. Pollinators are increasingly understood to constitute an integral part of agro-ecosystems and key to global sustainable productivity (Kevan 1999). The identification and management of rich bee plants and their integration into agricultural landscapes is recognized as a central component of bee conservation strategies (Delaplane and Mayer 2000).

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REFERENCES

Anonymous, 2003. Georgia annual crop summary, 2002. Georgia Farm Report 03, 2, p. 4.

Austin, M. 1994. Rabbiteye blueberries. Agscience, Inc. Auburndale, Florida.

Bertsch, A. 1983. Nectar production of *Epilobium angustifolium* L. at different air humidities: nectar sugar in individual flowers and the optimal foraging theory, Oecologia 59: 175-186.

Brewer, J.W., and R. C. Dobson. 1969. Nectar studies of the highbush blueberry *Vaccinium corymbosum* L., Cv. 'Rubel' and 'Jersey', HortScience 4: 332-334.

Cane, H.J., C. G. Eickwort, R. F. Wesley, and J. Spielholz. 1985. Pollination ecology of *Vaccinium stamineum* (Ericaceae: Vaccinioideae), Am. J. Bot. 72: 135-142.

Cane, J.H., and J.A. Payne. 1993. Regional, annual and seasonal variation in pollinator guilds: intrinsic traits of bees (Hymenoptera: Apoidea) underlie their patterns of abundance at *Vaccinium ashei* (Ericaceae). Ann. Entomol. Soc. Am. 86: 577-588

Corbet, S. A. 2003. Nectar sugar content: estimating standing crop and secretion rate in the field. Apidologie: 34: 1-10.

Corbet, S.A., D. M. Unwin, and O. E. Prys-Jones. 1979. Humidity, nectar and insect visits to flowers with special reference to *Crataegus, Tilia* and *Echium*, Ecol. Entomol. 4: 9-22.

Dafni, A. 1992. Pollination ecology: a practical approach. Oxford University Press Inc., New York.

Delaplane, K.S., and D. F. Mayer. 2000. Crop pollination by bees, CABI Publishing, Oxon, UK, 344 pp.

Dogterom, M.H., and M. L. Winston. 1999. Pollen storage and foraging by honey bees (Hymenoptera: Apidae) in highbush blueberries (Ericaceae), cultivar bluecrop. Can. Entomol. 131: 757-768.

Eck, P. 1988. Blueberry science. Rutgers University Press, New Brunswick, NJ, USA.

Eck, P., R. E. Cough, I. V. Hall, and J. M. Spiers. 1990. Blueberry Management. *In* Small fruit crop management. Prentice-Hall, Inc. NJ, USA, pp. 273-333.

Finnamore, A.T., and M E. Neary. 1978. Blueberry pollinators of Nova Scotia, with a checklist of the blueberry pollinators in eastern Canada and north-eastern United States. Ann. Soc. Entomol. Quebec, 23: 168-181.

France, L., C. M. S. Plowright, and R. C. Plowright. 2000. Behaviour of individual bumble bees (*Bombus impatiens*) on blueberry (*Vaccinium angustifolium*) flowers: the effects of pollen and nectar deprivation. J. Apic. Res. 39: 29 –35.

Gary, E.N. 1992. Activities and behavior of honey bees, *In* The hive and the honey bee. Dadant and Sons, Hamilton, Illinois, USA, pp. 269 -373.

Gilbert, F.S., N. Haines, and K. Dickson. 1991. Empty flowers. Funct. Ecol. 5: 29-39.

Horton, D., P. Bertrand, and G. Krewer. 1989. Small fruit pest management and culture, Bulletin 1022. Cooperative Extension Service, University of Georgia, GA, USA.

Harrison, F.H., and J. H. Fewell. 2002. Environmental and genetic influences on flight metabolic rate in the honey bee, *Apis mellifera*, Comp. Biochem. Phys. A, 133: 323 -333.

Heinrich, B. 1979. Bumblebee economics, Harvard University Press. USA.

Jablonski, B., S. Krol, K. Pliszka, and Z. Zurowska. 1985. Nectar secretion and pollination of the blueberry (*Vaccinium corymbosum* L.), Acta Hortic. 165: 133-144.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for the pollination biologist, University Press of Colorado, USA.

Kevan, G.P. 1999. Pollinators as bioindicators of the state of the environment: species, activity and diversity, Agric. Ecosyst. Environ. 74: 373-393.

Lang, G.A., and G. R. Danka. 1991. Honey-bee-mediated cross-versus self-pollination of 'Sharpblue' blueberry increases fruit size and hastens ripening, J. Am. Soc. Hortic. Sci. 116: 770-773.

Lloyd, S., D. J. Ayre, and R. J. Whelan. 2002. A rapid and accurate visual assessment of nectar production can reveal patterns of temporal variation in *Banksia ericifolia* (Proteaceae), Aust. J. Bot. 50: 595-600.

Mallick, S.A. 2001. Production and consumption of nectar in flowers of Tasmanian leatherwood, *Eucryphia lucida* (Eucryphiaceae), Aust. J. Bot. 49: 435-442.

Morrisette, R., A. Francoeur, and J-M. Perron. 1985. Importance des abeilles sauvages (Apoidea) dans la pollinisation des bleuetiers nains (*Vaccinium* spp.) en Sagamie, Quebec, Rev. Entomol. Quebec, 30: 44-53.

NeSmith, D.S. 1999. Blueberry research at the University of Georgia, 1999 research annual update. Research report 662.

Pleasants, J.M. 1983. Nectar production in *Ipomopsis aggregata* (Polemoniaceae), Am. J. Bot. 70: 1468-1475.

Pyke, G.H. 1978. Optimal foraging of bumblebees and coevolution with their plants, Oecologia, 36: 281-293.

Real, L.A., and B. J. Rathcke. 1991. Individual variation in nectar production and its effect on fitness in *Kalamia latifolia*, Ecology: 72: 149-155.

Rogers, A.H., and D. W. Gordon. 1992. Crop pollination, *In* The hive and the honey bee, Dadant and Sons, Hamilton, Illinois, USA, pp. 1043 - 1082.

Sampson, B. and J. Cane. 2000. Pollination efficiencies of three bee (Hymenoptera: Apoidea) species visiting rabbiteye blueberry. J. Econ. Entomol. 93: 1726-1731.

SAS Institute. 1992. SAS/STAT user's guide, version 8, SAS Institute; Cary, NC, USA, 846 pp. (4^{th} edition).

Seeley, D.T. 1985. Honey bee ecology, Princeton University Press, Princeton, NJ, USA.

Shuel, R.W. 1992. The production of nectar and pollen, *In* The hive and the honey bee, Dadant and Sons, Hamilton, Illinois, USA, pp. 401-436.

Southwick, E.E., G. M. Loper, and S. E. Sadwick. 1981. Nectar production, composition, energetics and pollinator attractiveness in spring flowers of western New York, Am. J. Bot. 68: 994-1002.

Spiers, J.M. 1978. Effect of stage of bud development on cold injury in rabbiteye blueberry, J. Am. Soc. Hortic. Sci. 103: 452-455.

Thomson J. D. (1988) Effects of variation in inflorescence size and floral rewards on the visitation rates of traplining pollinators of *Aralia hispida*, Evol. Ecol. 2, 65-76.

Wesselingh, R.A., and M. L. Arnold. 2000. Nectar production in Louisiana iris hybrids, Int. J. Plant. Sci.161(2): 245-251.

Williams, S.C. 1997. Nectar secretion rates, standing crop and flower choice by bees on *Phacelia tanacetifolia*, J. Apic. Res. 36: 23-32.

Winston, M. L. 1987. The biology of the honey bee, Harvard University Press, Cambridge, Mass. USA.

Wood, G.W. 1961. The association between age of inflorescence and nectar production in the low-bush blueberry *Vaccinium angustifolium*, Can. J. Bot. 39: 1037-1040.

Table 2.1. 24-h nectar production as affected by age of flower. Day 1 is day of anthesis. Values are means ± standard error, with *n* in parentheses. Means within a column followed by the same letter are not significantly different at the $\alpha = 0.05$ level.

Flower age (day)	Quantity of sucrose (mg / flower)	Brix (%)	Volume of nectar (µl / flower)
1	0.16 ± 0.04 (20)b	16.5 ± 0.48 (20)b	0.85 ± 0.18 (20)a
2	0.47 ± 0.12 (20)ab	27.30 ± 0.73 (20)a	1.53 ± 0.40 (20)a
3	0.76 ± 0.19 (20)a	28.33 ± 2.20 (20)a	2.21 ± 0.45 (20)a
4	0.65 ± 0.20 (17)a	28.4 ± 2.36 (17)a	1.86 ± 0.49 (17)a

Table 2.2. Nectar standing crop in blueberry rabbiteye flowers. Values are means ± standard error, with *n* in parentheses. Means within a column followed by the same letter are not significantly different at the $\alpha = 0.05$ level.

Flower age (day)	Quantity of sucrose (mg / flower)	Brix (%)	Volume of nectar (µl / flower)
One day	0.28 ± 0.03 (35)c	19.51 ± 1.67 (35)c	0.90 ± 0.11 (65)b
Two days	1.01 ± 0.07 (119)b	31.22 ± 1.15 (119)b	2.46 ± 0.14 (140)a
Older then two days	1.37 ± 0.07 (163)a	36.66 ± 0.98 (163)a	2.79 ± 0.14 (191)a
Total	1.09 ± 0.04 (439)	29.45 ± .64 (439)	2.85 ± 0.1 (530)

Figure 2.1. Flowers of rabbiteye blueberry (a) at day of anthesis (day 1), (b) day 2 and (c) > 2 d old. Ink marks on the florets were used by the investigator to track age of florets. The corollae of one-day old flowers (a) are strongly wrinkled and their apertures narrowly opened, while in two-day old flowers (b) corollae are more weakly wrinkled and apertures more expanded. In flowers > two days (c) the corollae are fully rounded and apertures maximally expanded.

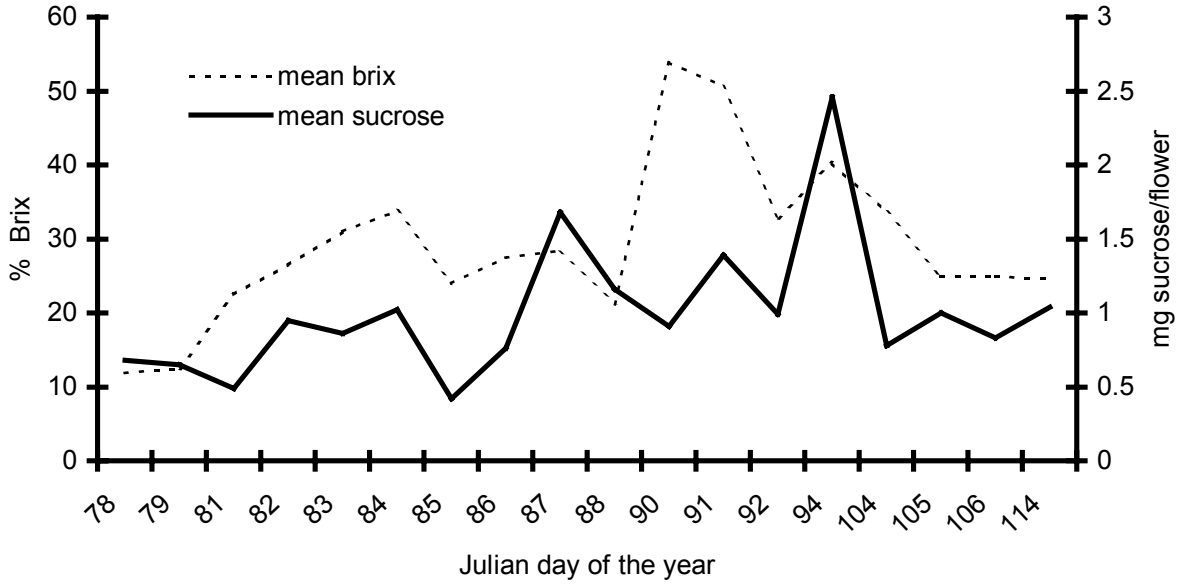

Figure 2.2. The average quantity of nectar standing crop (mg / flower) and sucrose concentration (% Brix) of rabbiteye blueberry flowers during blooming season**.**

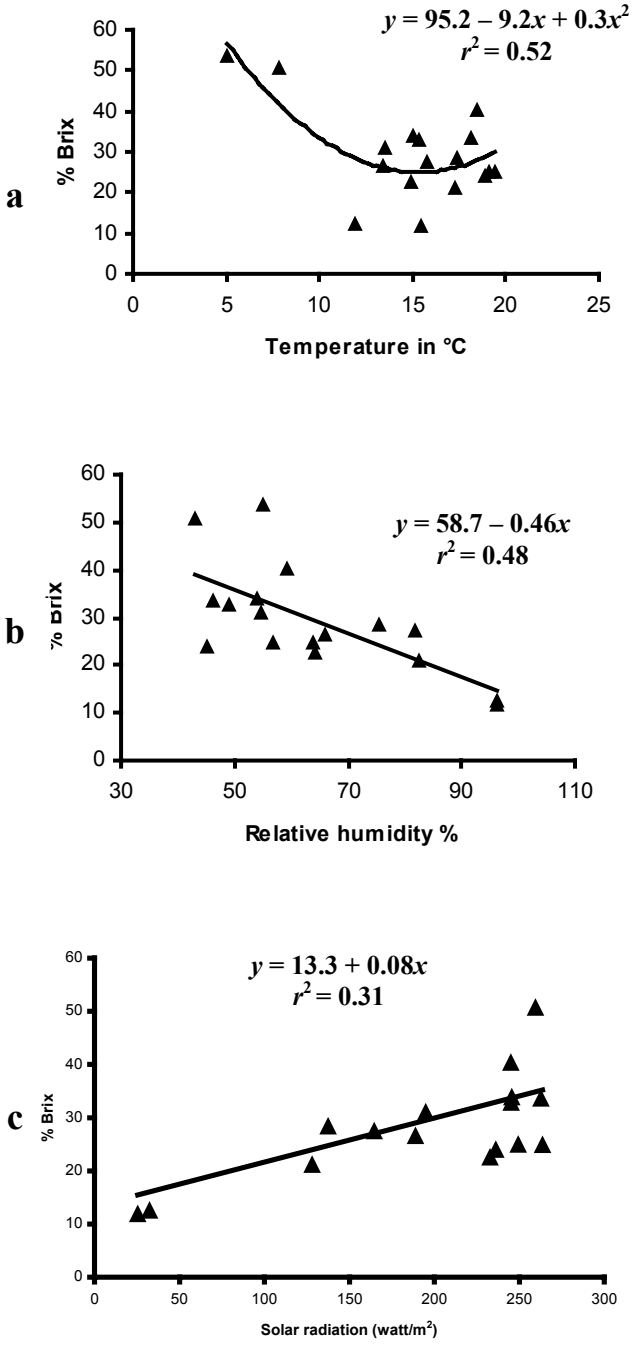

Figure 2.3. Relationship of sucrose concentration (% Brix) in nectar with average (a) air temperature (°C), (b) relative humidity and (c) solar radiation observed during 24-hour preceding measurements. Observed values are mean sucrose concentration (▲), and the line connects predicted values.

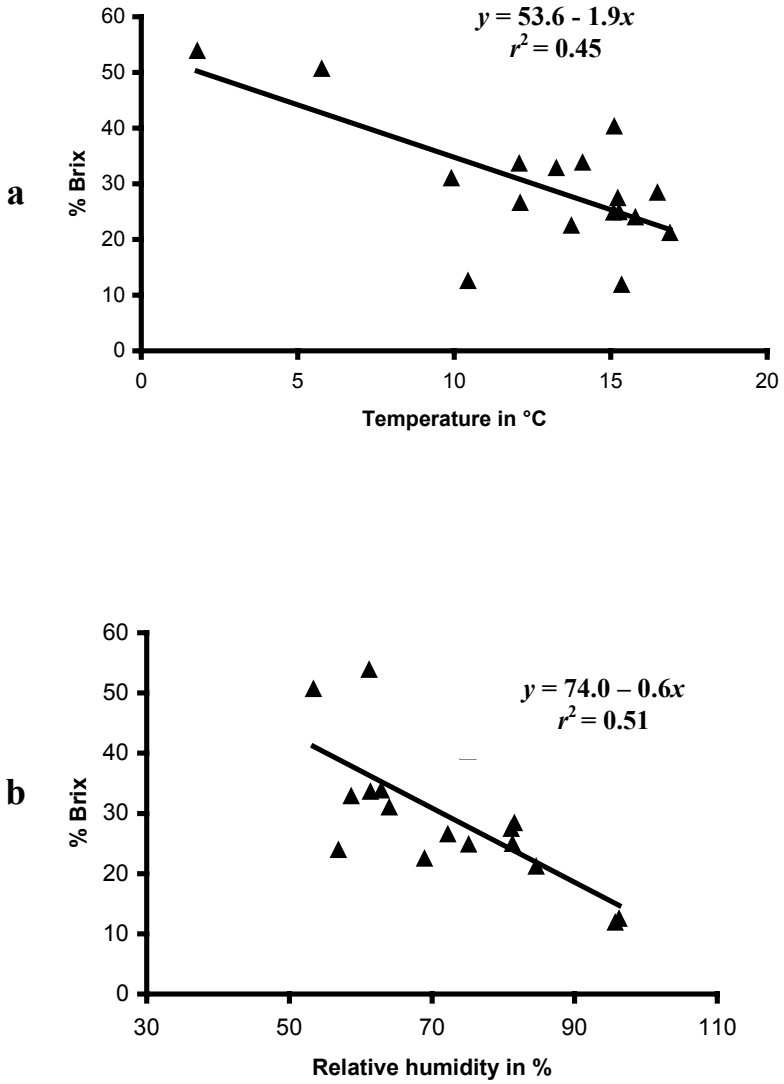

Figure 2.4. Relationship of sucrose concentration (% Brix) in nectar with average (a) air temperature (°C) and (b) relative humidity observed during 12-hours preceding measurements. Observed values are mean sucrose concentration (▲), and the line connects predicted values.

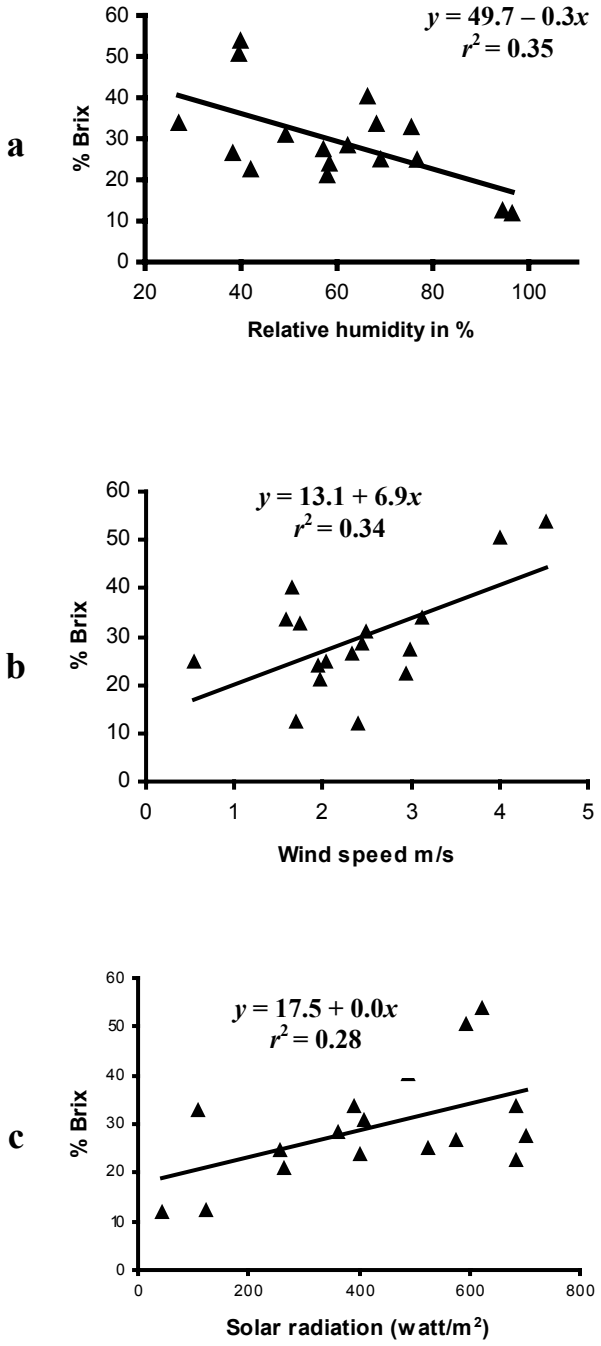

Figure 2.5. Relationship of sucrose concentration (% Brix) in nectar with average (a) relative humidity, (b) wind speed and (c) solar radiation (watt/m^2) recorded at time of measurements. Observed values are mean sucrose concentration (▲), and the line connects predicted values.

CHAPTER 3

HONEY BEE (HYMENOPTERA : APIDAE) POLLINATION OF RABBITEYE BLUEBERRY *VACCINIUM ASHEI* VAR. 'CLIMAX' IS POLLINATOR DENSITY-DEPENDENT[1]

[1] Dedej S. and K. S. Delaplane. 2003. *Journal of Economic Entomology*. 96 (4): 1215-1220.
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ABSTRACT

In a 2-year field study, mature orchard plants of rabbiteye blueberry (*Vaccinium ashei* Reade var. 'Climax'), plus potted pollenizers ('Premier') were caged with varying densities of honey bees (0, 400, 800, 1,600, 3,200, 6,400 or 12,800 bees plus open plot) during the bloom interval. The rate of legitimate flower visits tended to increase as bee density increased within a range of 400 – 6,400 bees; there were more legitimate visits in cages with 6,400 bees than in those with ≤1,600 bees. Similarly, within a range of 400 – 6,400 bees there was a trend for a corresponding increase in fruit-set with means ranging from 25.0 to79 percent. Fruit-set was higher in cages with 6,400 or 3,200 bees than in those with ≤800 bees. Regression analyses showed that fruit-set increased linearly with the rate of legitimate bee visits. Mean weight of berries was unaffected by bee density but varied significantly between years. Within a range of 0 – 3,200 bees/cage the average seeds per berry tended to increase with increasing bee density; there were more seeds in open plots than in cages with 12,800 honey bees or ≤1,600 bees. Sucrose content ranged from 12.1 to16.7 percent and fruits tended to have more sugar in cages with lower bee densities. Speed of ripening tended to be higher in cages with higher bee densities. Earlier work has shown that the effectiveness of *Apis mellifera* L. as a pollinator of rabbiteye blueberry is variety-dependent. Our data indicate that the effectiveness of *A. mellifera* is also bee density-dependent.

KEY WORDS pollination, rabbiteye blueberry, *Vaccinium ashei*, *Apis mellifera*, bee density

INTRODUCTION

Insect pollination is beneficial for blueberry production, especially for rabbiteye cultivars (*Vaccinium ashei* Reade) that are generally self-incompatible and require cross-pollination with

another rabbiteye cultivar (Delaplane and Mayer 2000). Rabbiteye blueberries often have low fruit-set and unacceptable commercial yields (Lyrene and Crocker 1983), problems usually associated with poor pollination (Filmer and Marucci 1963).

Honey bees (*Apis mellifera* L.) are the most numerous bee visitors of blooming rabbiteye blueberries in south Georgia, USA, followed in descending order of abundance by bumble bee queens (*Bombus* spp.), bumble bee workers, carpenter bees (*Xylocopa* spp.), and southeastern blueberry bees (*Habropoda laboriosa* (F.)) (Delaplane 1995). Based on single-bee flower visits, *H. laboriosa* and *Bombus* queens were determined to be the most efficient pollinators of rabbiteye variety 'Tifblue,' and honey bees the least efficient (Cane and Pane 1990). This disparity is due in part to the catholic foraging habits of honey bees, their inability to sonicate (Delaplane and Mayer 2000), and the comparative inaccessibility of the 'Tifblue' flower to short-tongued honey bees. Despite these disadvantages, honey bees are widely recognized as a valuable resource for blueberry growers (Dorr and Martin 1966) to ensure adequate blossom pollination (Eck 1988). They are easily managed and available in large numbers all year long. Moreover, the success with honey bees realized by Sampson and Cane (2000) with the rabbiteye variety 'Climax' suggests that the pollination efficacy of honey bees on *V. ashei* is variety-dependent.

We were further interested in whether the efficacy of honey bees is bee density-dependent. It is conceivable that an individually inefficient pollinator species, as measured by single-bee flower visits, may perform satisfactorily if it is able to field a forager force large enough to increase the rate of legitimate flower visits. Hence, in this 2-year study we evaluated the effects of different honey bee densities on the rate of legitimate bee flower visits, rabbiteye

blueberry fruit-set, berry weight, number of seeds, percent sucrose content of juice, and speed of ripening.

MATERIALS AND METHODS

Plants used for this experiment were part of a permanent orchard at the Horticulture Farm of the University of Georgia, Oconee County, GA, USA. The experiment was replicated in both 2000 and 2002 and consisted of caging plants with varying densities of honey bees during the bloom interval and then measuring certain characters of fruits. Each caged plot contained 4 rabbiteye blueberry plants (two mature 'Climax' plants with two potted 'Premier' as pollenizers). Cages were 1.8 x 1.8 x 1.8 m frames covered with Lumite screen (Bioquip., CA, USA). For each year, each 1.8 x 1.8 x 1.8 m plot was assigned one of eight experimental treatments: caged with one honey bee colony containing either 400, 800, 1,600, 3,200, 6,400 or 12,800 bees, containing zero bees, or left open as a positive control. Bees were added to the plots (10 March in 2000, 22 March in 2002) when advanced buds at stage five were still unopened (Spiers 1978). Target bee populations were reached by the gravimetric methods of Delaplane and Hood (1997). Open plots were not provided with potted pollenizers because the orchard was already planted in alternating rows of 'Climax' and 'Premier' plants. Because the apiary of University of Georgia was near the experimental field, honey bees, as well as other bee species, were able to freely visit the open plots. Plants were irrigated as we deemed necessary.

Bee colonies were fed regularly with sugar syrup and socially stabilized with synthetic queen mandibular pheromone (QMP) (one queen equivalent of Bee-Boost^{TM} [Phero Tech, BC, Canada]) (Currie et al. 1994). QMP was used in lieu of a queen to eliminate confounding effects of differential brood production resulting from variable bee populations.

After bloom was finished, the honey bee colonies were removed and final bee populations determined as before. The Lumite screens were removed to minimize shade effects and all plots netted with poultry fencing to protect fruit from animals and unauthorized harvesting.

The average density of honey bees ([beginning + ending] ÷ 2) for each cage was determined to be 245, 468, 1,008, 2,273, 4,186 and 8,468 (for the 400, 800, 1,600, 3,200, 6,400, and 12,800 bee groups respectively) for the year 2000 and 286, 609, 1,359, 2,228, 4,490 and 10,656 for 2002. These values were later used for regression analyses of continuous effects, but for convenience we discuss the discrete effects in terms of initial cage densities. The number of unopened florets per raceme was determined for 40 tagged racemes on the 'Climax' plants in each plot during 8-9 March 2000 and 18-20 March 2002 prior to inserting bee colonies into cages at the stage 4 and 5 of blooming (Spiers 1978). To compensate for wind loss of tags, we marked twice as many racemes (80) in the open plots in 2002.

On several days during the bloom period (early March – early April), we measured for each plot (excepting the 0 bee plots) the rate of legitimate honey bee flower visits (number of legitimate flower visits per 2 min) during normal flight hours (11.00 – 16.00). Visits during which honey bees probe the terminal aperture of the flower, presumably effecting pollination, were considered legitimate whereas those realized by probing lateral robbery holes in corollae made by carpenter bees (*Xylocopa virginica* (L.)) were considered illegitimate (Faegri and Van der Pijl 1979). *Xylocopa virginica* is ubiquitous in the Southeast and with *V. ashei* it invariably engages in nectar thievery. Honey bees are incapable of making robbery holes but readily visit holes made by *X. virginica* and thus act as secondary nectar thieves (Delaplane and Mayer 2000).

Harvest of fruits started at the beginning of June and finished about the middle of July depending on the year. The following dependent variables were measured for each recovered raceme: percent fruit-set, number of seeds per fruit, berry weight (g), speed of ripening, and sucrose content of juice. Percentage fruit-set ([no. fully-formed fruit ÷ no. unopened florets] x 100) was determined for each raceme with ripe fruit (2000) or full-sized green fruit (2002). Number of seeds per fruit was determined by expressing berry contents and counting the number of fully-formed seeds. Speed of ripening was calculated for only one year as the percentage of fruits ripe on one arbitrarily-chosen date: June 1, 2002. Percent sucrose content of juice was determined with a bench-top refractometer (Fisher, PA, USA).

Because independent variables used in this study were both discrete (bee density) and continuous (rate of legitimate flower visits, log average bee density), we used both analysis of variance (ANOVA) and regression models. The effects of bee density on rate of legitimate bee visits, fruit-set, berry weight, seeds per berry, and percent sucrose content were determined with a completely randomized analysis of variance (ANOVA) blocked on year and recognizing density x year interaction as test term. Speed of ripening was measured only one year, so its analysis employed residual error as test term and was not blocked. Means were separated by Duncan's test, and differences were accepted at the $\forall \leq 0.05$ level (SAS Institute 1992). The relationships of fruit-set and seeds per berry with log-transformed average bee population ($\log_{10}$ [avg. population + 1]) as well as the relationships of fruit-set and seeds per berry with rate of legitimate bee visits, and g per berry with seeds per berry were analyzed with regression models testing for linear, quadratic and cubic effects (SAS Institute 1992).

RESULTS AND DISCUSSION

Bee density effects on flower visitation. There were bee density effects for the rate of legitimate bee visits (F=4.4; df=6,6; P=0.0463), but no year effects (F=0.1; df=1,6; P=0.736). Rate of visitation tended to increase with increasing bee density up to 6,400 bees (Table 3.1). These results are important because they support the general assumption that increases in *A. mellifera* density correspond to actual increases in flower visitation.

Fruit-set. Fruit-set was affected by bee density (F=5.2; df=7,7; P=0.0223), but not year (F=3.0; df=1,7; P=0.1284). Within a range of 400 – 6,400 honey bees, there was a trend for increasing fruit-set as bee number increased (Table 3.1). The highest fruit-set was achieved in the open plots and in cages with ≥ 1,600 honey bees. When fruit-set was analyzed as a response variable related to log-transformed average bee density, the best fit was achieved with a quadratic model (Fig. 3.1). Declining fruit-set at upper bee density levels is best explained as an artifact of experimental conditions; bees in the 12,800 plots frequently exhibited aberrant flight behavior (flying against the screens) and it is possible that the comparatively high bee : flower ratio reduced the average pollen load of individual foragers. When fruit-set was analyzed as a response variable and related to rate of legitimate bee visits (Fig. 3.2), the resulting best-fit model was linear, indicating no leveling off at upper rates of visitation. It remains to be determined the rate of flower visitation beyond which no additional benefit to fruit-set is realized.

The effectiveness of *A. mellifera* as a pollinator of rabbiteye blueberry is partly variety-dependent. Honey bees were demonstrated to be inefficient pollinators of ‘Tifblue’ (Cane and Payne 1990) but effective for ‘Climax’ (Sampson and Cane 2000) based on assays of single-bee flower visits. Our results support those of Sampson and Cane (2000) and confirm that *A.*

mellifera is an effective pollinator of *V. ashei* var. 'Climax.' Our data further indicate that the effectiveness of *A. mellifera* is bee density-dependent. More broadly, our results underscore the need to consider the pollinator densities achievable with candidate pollinator species. It is possible that a relatively inefficient pollinator, as determined by a lack of specialized behaviors or phenologies, may nevertheless be effective if it can field a forager force large enough to increase the rate of legitimate flower visitation.

The general manageability of honey bees, considered with their demonstrated variety-specific efficacy as pollinators of *V. ashei* (present results, Sampson and Cane 2000) argue for renewed attention to the plant side of the pollination management syndrome. It is plausible to select for floral morphology in *V. ashei* that is conducive to honey bee pollination. Lyrene (1994) suggested that comparatively short and wide corollae, large apertures, and short distances between stigmata and anthers are favorable characters that would make the blueberry flower more amenable to honey bee pollination. Flowers of *V. ashei* in general exhibit the unfavorable end of these spectra, although there is inter-varietal variation. Indeed, the difference in honey bee efficacy noted between 'Tifblue' (Cane and Payne 1990) and 'Climax' (present results, Sampson and Cane 2000) are likely traced to the comparative shortness of the 'Climax' flower. A genetic solution to this problem was intimated by Ritzinger and Lyrene (1999) who were able to show that F_1 hybrids between *V. ashei* and a wild subspecies expressing desirable flower characteristics, *V. constablaei*, displayed flower characteristics intermediate between the two. Such genetic plasticity suggests that plant breeders could select for *V. ashei* phenotypes that are more conducive to honey bee pollination.

Seed Number. The number of fully developed seeds per berry was affected by bee density (F=7.2; df=7,7; P=0.0093), but not year (F=0.3; df=1,7; P=0.6223). The number of seeds

is a good indicator of the effectiveness of the pollinator as well as a measure of female fertility if compatible pollen is abundant (Ritzinger and Lyrene 1998). In our study the number of seeds per berry was highest in the open plots and plots with 3,200 or 6,400 honey bees; among caged plots there was a tendency for increasing seed number as bee density increased (Table 3.1). These results are consistent with previous studies that found that blueberry fruits produce few seeds when insect pollinators are excluded (Lang and Danka 1991, Froborg 1996, Sampson and Cane 2000).

When seed number was analyzed as a response variable to log-transformed average bee density, the best fit was achieved with a quadratic model (Fig. 3.3). Declining seed numbers at upper bee density levels is best explained as an artifact of experimental conditions as described above for fruit-set. When seed number was analyzed as a response variable to rate of legitimate bee visits (Fig. 3.4), the resulting best-fit model was linear, indicating no leveling off at upper levels of visitation, similar to the results for fruit-set. Indeed, one of the most notable features of our study is the general congruence of trends for fruit-set and seed number (compare, for example, Figs. 3.1 and 3.3 with Figs. 3.2 and 3.4).

One possible exception to this trend for congruence is the conspicuously high seed numbers achieved in the open plots (Table 3.1). One explanation for this is the likelihood that flower visitation by a diversity of bee species optimizes seed-set. *Vaccinium* spp. are responsive to sonicating pollinators such as *Bombus* spp. and *H. laboriosa* (Delaplane and Mayer 2000), and we observed sonicating *Bombus* spp. visiting open plots during this study. Another explanation is the relative abundance of pollenizer pollen in open plots. The high fruit-set achieved in caged plots with relatively intermediate seed numbers (Table 3.1), however, suggests a partial physiological independence between seed-set and fruit-set in *V. ashei* var. 'Climax.'

Berry Weight. Average berry weight was affected by year (F=23.0; df=1,7; P=0.002), but not bee density (F=0.9; df=7,7; P=0.564). Average weight (g) of berries was higher in 2002 (1.35 ± 0.03, 0 ± SE, n=240) than in 2000 (0.75 ± 0.02, n=203). When berry weight was tested as a response variable against seed number the best fit was achieved with a quadratic model (Fig. 3.5) wherein berry weight responded positively with seed number but leveled off and declined at upper levels. A positive relationship between the two is expected in *Vaccinium* spp. (Filmer and Marucci 1963, Dorr and Martin 1966, Brewer and Dobson 1969, Moore et al. 1972) but is not universal, as demonstrated by MacKenzie (1997), who detected no association between seed number and fruit weight in highbush blueberry. With rabbiteye there appears to be a correspondence between small fruit size and low seed number in plants treated with the growth regulator gibberellic acid (NeSmith et al. 1995). Our data implicate a physiological limit to the responsiveness of fruit development to seed set in rabbiteye.

Sucrose Content. The percent sucrose content of juice was affected by bee density (F=5.4; df=7,7; P=0.0201) and year (F=13.3; df=1,7; P=0.0082). Fruit juice tended to have more sucrose in plots with fewer bees and correspondingly lower fruit-set (Table 3.1). Fruits from plants experiencing poor fruit-set are invested with a comparatively higher fraction of available carbohydrates. The percentage sucrose was higher in 2002 (14.8 ± 0.2%) than 2000 (13.3 ± 0.2%). The most notable climatic difference between years was greater rainfall in 1 January – 31 July 2002 (Γ=586.5 mm) than for the same period in 2000 (451.4 mm).

Speed of Ripening. Speed of fruit ripening was affected by honey bee density (F=9.1; df=7, 274; P< 0.0001). Fruits ripened more quickly in plots with ≥ 3,200 bees than in plots with 1,600 bees or ≤ 400 (Table 3.1). Speed of ripening was higher in the 3,200-bee plot than in the open plot. Cross-pollination is known to improve speed of ripening in highbush blueberries

(MacKenzie 1997); the present data indicate a similar benefit when pollination is optimized in rabbiteye.

Conclusion. Earlier work has shown that the effectiveness of *Apis mellifera* as a pollinator of rabbiteye blueberry is variety-dependent. Our data indicate that *V. ashei* var. 'Climax' responds positively to increases in honey bee density as measured by fruit-set, seed number, and speed of ripening. We conclude that honey bee pollination of *V. ashei* var. 'Climax' is also pollinator density-dependent.

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REFERENCES

Brewer, J.W. and R.C. Dobson. 1969. Seed count and berry size in relation to pollinator level and harvest date for the highbush blueberry, *Vaccinium corymbosum*. J. Econ. Entomol. 62(6): 1353-1356.

Cane, J.H. and J.A. Payne. 1990. Native bee pollinates rabbiteye blueberry. Alabama Agric. Exp. Sta. 37(4): 4.

Currie, R.W., M.L. Winston, and N.K. Slessor. 1994. The effect of honey bee (*Apis mellifera* L.) synthetic queen mandibular compound on queenless 'Disposable' pollination units. Am. Bee J. 134: 200-202.

Delaplane, K.S. 1995. Bee foragers and their pollen loads in South Georgia rabbiteye blueberry. Am. Bee J. 135: 825-826.

Delaplane, K.S. and W.M. Hood. 1997. Effects of delayed acaricide treatment in honey bee colonies parasitized by *Varroa jacobsoni* and a late-season treatment threshold for the south-eastern USA. J. Apic. Res. 36(3/4): 125-132.

Delaplane, K.S. and D.F. Mayer. 2000. Crop pollination by bees. CABI Publishing, Oxon, UK, 344 pp.

Dorr, J. E. and E. C. Martin. 1966. Pollination studies on the highbush blueberry, *Vaccinium corymbosum* L. Mich. Agric. Exp. Sta. Quart. Bull. 48: 437-448.

Eck, P. 1988. Blueberry science. Rutgers University Press, New Brunswick, NJ, USA.

Faegri, K. and L. W. Van der Pijl. 1979. The principles of pollination ecology (third edition). Pergamon, Oxford, UK, 244 pp.

Filmer, R.S. and P.E. Marucci. 1963. The importance of honeybees in blueberry pollination. 31st Ann. Blueberry Open House Proc., North Carolina Agric. Exp. Sta., pp. 14-21.

Froborg, H. 1996. Pollination and seed production in five boreal species of *Vaccinium* and *Andromeda* (Ericaceae). Can. J. Bot. 74: 1363-1368.

Lang, G.A. and G.R. Danka. 1991. Honey-bee-mediated cross-versus self-pollination of 'Sharpblue' blueberry increases fruit size and hastens ripening. J. Am. Soc. Hortic. Sci. 116: 770-773.

Lyrene, P.M. 1994. Variation within and among blueberry taxa in flower size and shape. J. Am. Soc. Hortic. Sci. 119: 1039-1042.

Lyrene, P.M. and T.E. Crocker. 1983. Poor fruit set on rabbiteye blueberries after mild winter: Possible causes and remedies. Proc. Florida State Hortic. Soc. 96: 195-197.

MacKenzie, E.K. 1997. Pollination requirements of three highbush blueberry (*Vaccinium corymbosum* L.) cultivars. J. Am. Soc. Hortic. Sci. 122: 891-896.

Moore, J.N., B.D. Reynolds, and G.R. Brown. 1972. Effects of seed number, size, and development on fruit size of cultivated blueberries. HortScience 7: 268-269.

NeSmith, D.S., G. Krewer, M. Rieger, and B. Mullinix. 1995. Gibberellic acid-induced fruit set of rabbiteye blueberry following freeze and physical injury. HortScience 30: 1241-1243.

Ritzinger, R. and P.M. Lyrene. 1998. Comparison of seed number and mass of southern highbush blueberries vs. those of their F_1 hybrids with *V. simulatum* after open pollination. HortScience 33: 887-888.

Ritzinger, R. and P.M. Lyrene. 1999. Flower morphology in blueberry species and hybrids. HortScience 34:130-131.

Sampson, B. and J. Cane. 2000. Pollination efficiencies of three bee (Hymenoptera: Apoidea) species visiting rabbiteye blueberry. J. Econ. Entomol. 93: 1726-1731.

Spiers, J.M. 1978. Effect of stage of bud development on cold injury in rabbiteye blueberry. J. Am. Soc. Hortic. Sci. 103: 452-455.

SAS Institute 1992. SAS/STAT user's guide, version 6. SAS Institute; Cary, NC, USA, 846 pp. (4^{th} edition).

Table 3.1. Bee flower visitation and fruit characteristics of 'Climax' rabbiteye blueberry as affected by honey bee density in cages (ca. 2x2 m). Data are pooled for years 2000 and 2002 except for speed of ripening which was determined only for 2002. Values are means ± standard errors, with n in parentheses. Means within a column followed by the same letter are not significantly different at the $\alpha = 0.05$ level.

Honey bee density	Legitimate bee visits / 2 min	Fruit set (%)	Mature seeds / berry	Berry weight (g)	Sucrose content of juice (%)	Speed of ripening (%)
Open plot	2.3 ± 0.8 (21)cd	68.9 ± 3.1 (98)ab	23.1 ± 1.3 (63)a	1.2 ± 0.1 (64)a	12.0 ± 0.2 (63)c	27.2 ± 3.5 (67)b
No bees	NA	32.4 ± 3.5 (67)c	0.2 ± 0.1 (43)c	0.8 ± 0.05 (43)a	15.9 ± 0.3 (43)ab	11.5 ± 4.8 (25)c
400	0.5 ± 0.3 (22)d	25.0 ± 3.3 (68)c	1.0 ± 0.5 (34)c	0.9 ± 0.05 (33)a	16.7 ± 0.5 (34)a	9.9 ± 3.7 (18)c
800	4.9 ± 1.4 (21)bcd	48.7 ± 4.1 (78)bc	6.9 ± 1 (57)bc	1.1 ± 0.1 (57)a	16.0 ± 0.4 (57)ab	28.8 ± 5.4 (35)b
1600	7.8 ± 2.4 (21)bcd	52.4 ± 3.5 (70)abc	8.1 ± 0.8 (60)bc	0.9 ± 0.04 (61)a	13.2 ± 0.4 (59)c	8.0 ± 2.5 (32)c
3200	20.3 ± 2.9 (21)ab	79.1 ± 3.3 (63)a	14.2 ± 0.9 (62)ab	1.2 ± 0.1 (62)a	13.1 ± 0.3 (62)c	49.3 ± 5.1 (36)a
6400	25.5 ± 3.4 (20)a	79.0 ± 2.9 (71)a	14.1 ± 0.8 (64)ab	1.2 ± 0.1 (66)a	13.8 ± 0.3 (65)bc	37.1 ± 4.8 (36)ab
12800	16.4 ± 2.1 (22)abc	52.2 ± 4.0 (67)abc	6.5 ± 0.6 (57)bc	1.1 ± 0.1 (57)a	13.7 ± 0.4 (56)bc	38.2 ± 5.5 (33)ab

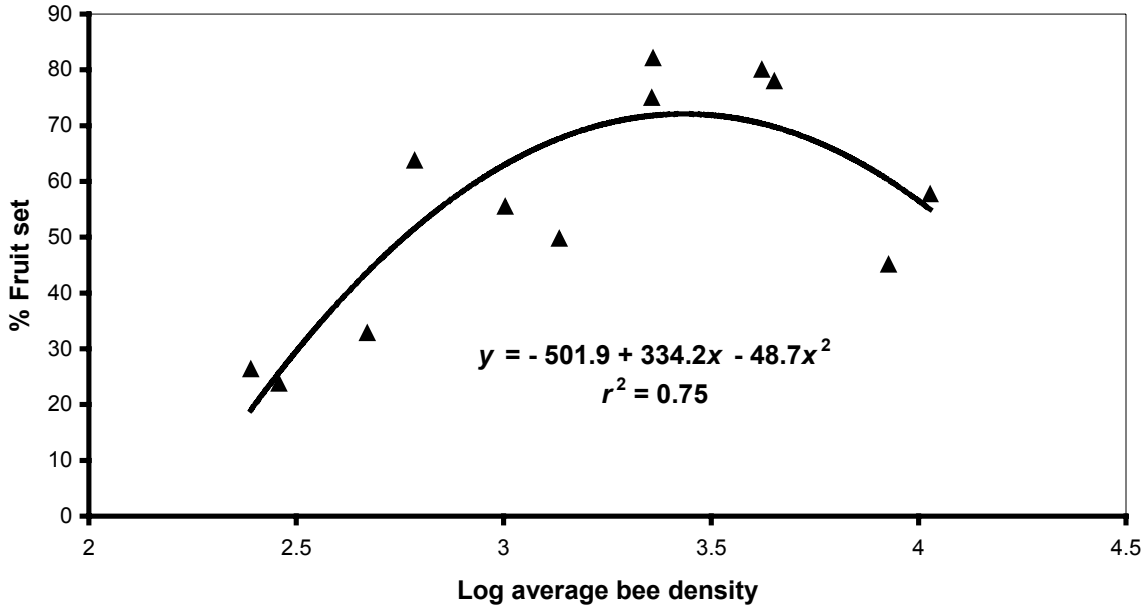

Fig. 3.1. Regression of fruit set in 'Climax' rabbiteye blueberry with honey bee density in field cages (expressed as log average density). Observed values are mean fruit set (▲), and the line connects predicted values from the quadratic model.

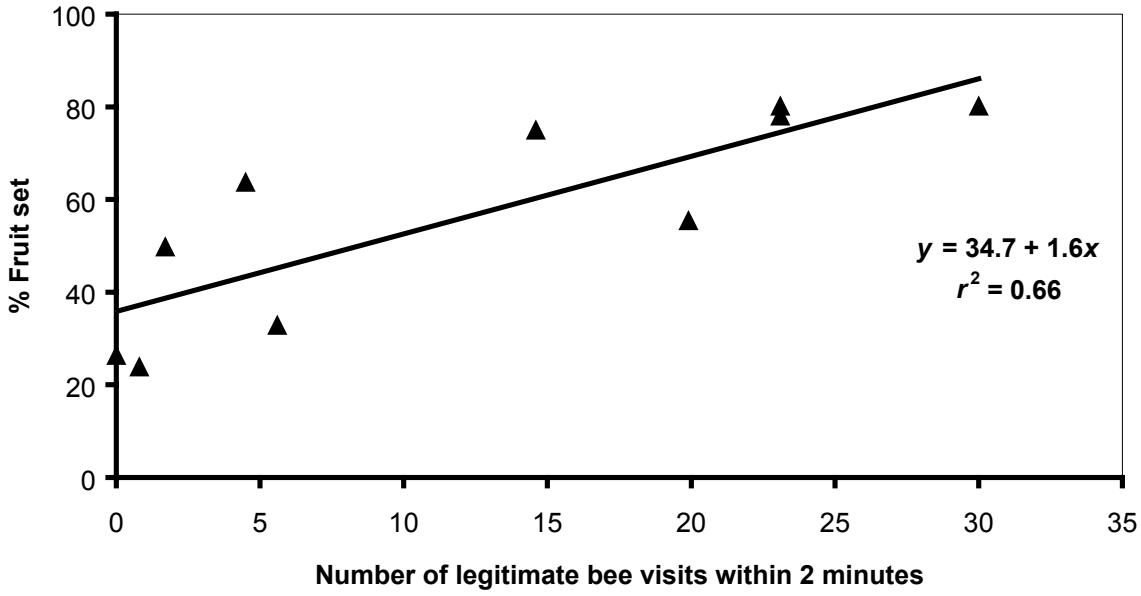

Fig. 3.2. Regression of fruit set in 'Climax' rabbiteye blueberry in relation to the number of legitimate bee visits within two minutes in field cages. Observed values are mean fruit set (▲), and the line connects predicted values from the linear model.

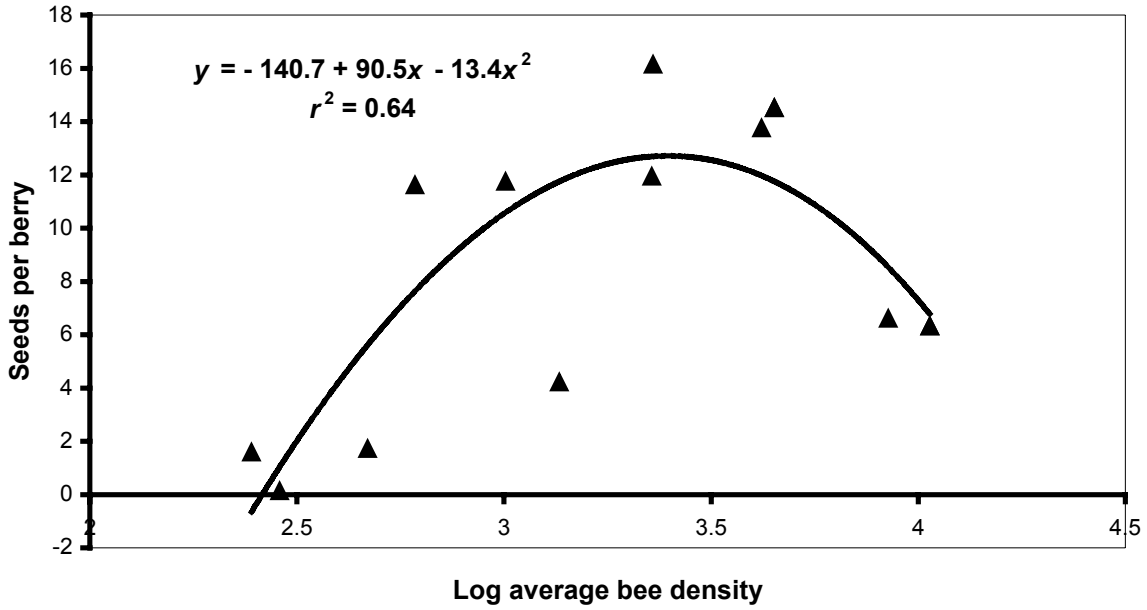

Fig. 3.3. Regression of number of seeds per berry in 'Climax' rabbiteye blueberry with honey bee density in field cages (expressed as log average density). Observed values are mean seeds per berry (▲), and the line connects predicted values from the quadratic model.

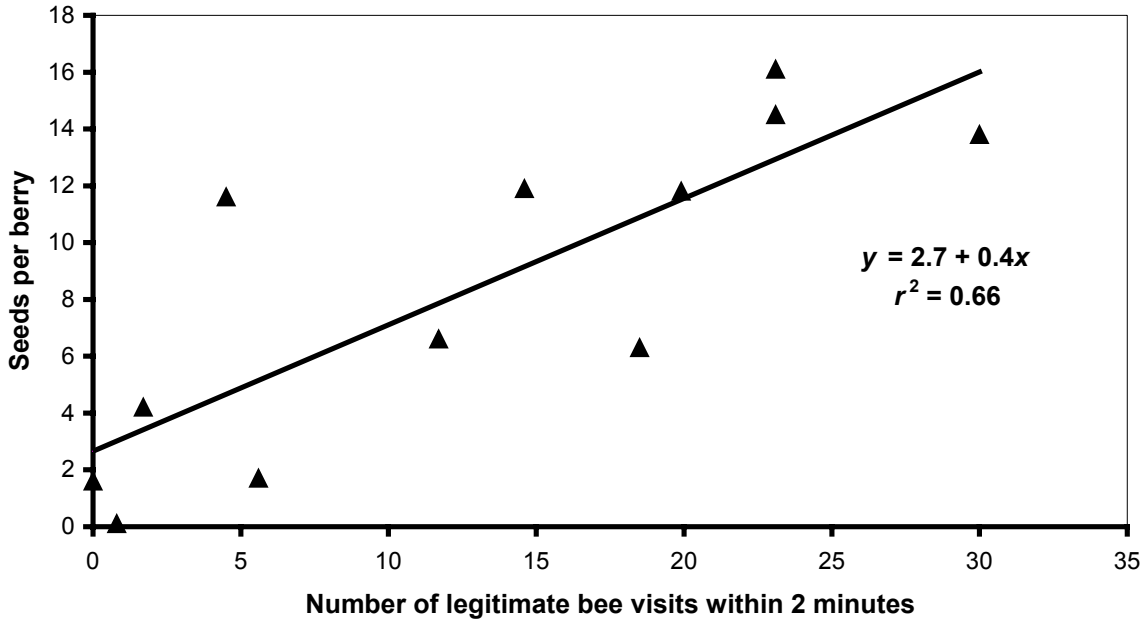

Fig. 3.4. Seed number in 'Climax' rabbiteye blueberry in relation to the number of legitimate bee visits within two minutes in field cages. Observed values are mean seeds per berry (▲), and the line connects predicted values from the linear model.

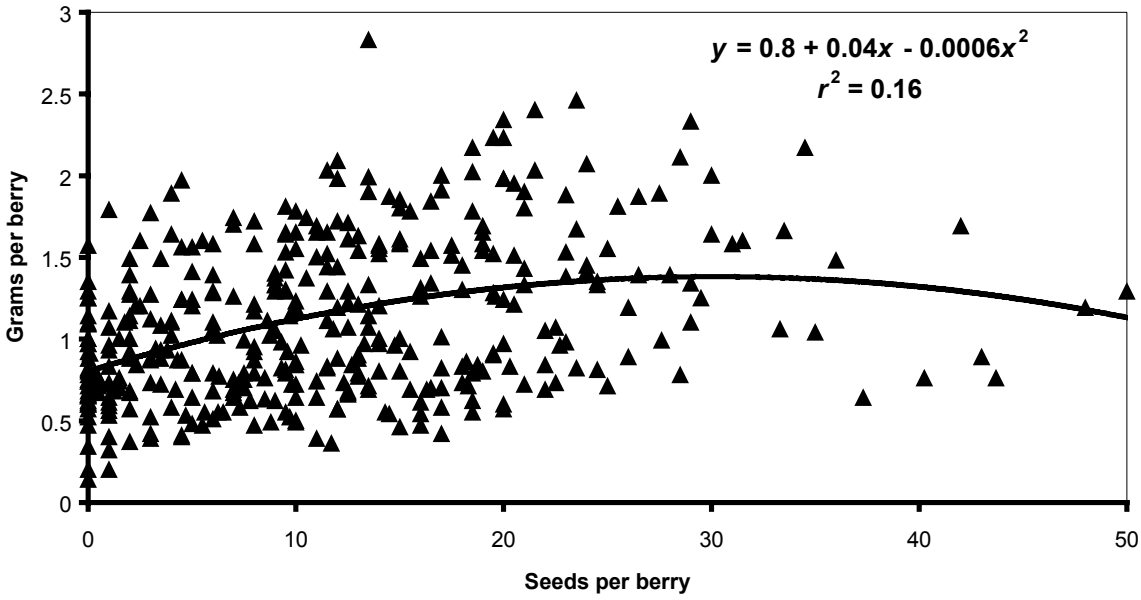

Fig. 3.5. Berry weight (g) in 'Climax' rabbiteye blueberry in relation to number of fully-formed seeds. Observed values are berry weight (▲), and the line connects predicted values from the quadratic model.

CHAPTER 4

NECTAR-ROBBING CARPENTER BEES REDUCE SEED-SETTING CAPABILITY OF HONEY BEES (HYMENOPTERA : APIDAE) IN RABBITEYE BLUEBERRY *VACCINIUM ASHEI* READE VAR. 'CLIMAX'[1]

[1] Dedej S. and K. S. Delaplane. 2004. *Journal of Environmental Entomology*. 33 (1): 100-106. Reprinted here with permission of publisher, 05/10/2004.

ABSTRACT

The carpenter bee *Xylocopa virginica* (L.) acts as a primary nectar thief in Southeastern plantations of native rabbiteye blueberry *Vaccinium ashei* Reade, perforating corollae laterally to imbibe nectar. Honey bees (*Apis mellifera* L.) learn to collect nectar from these perforations and thus become secondary thieves. We conducted a 2-yr study to assess how nectar robbing in honey bees affects fruit production in rabbiteye blueberry. Various harvest parameters were measured from fruit collected from plants tented with: honey bees and carpenter bees (AX), carpenter bees (X), honey bees (A), no bees (0), or in open plots (open). In open plots, rates of illegitimate honey bee flower visitation increase from initial lows to fixation at ≥95%. Fruit-set is higher in open, A, and AX plots than in X and 0 plots. Even though fruit-set is similar in A and AX plots, seed numbers are significantly reduced in AX plots in which *X. virginica*-induced illegitimate honey bee flower visitation approaches 40%. Open-pollinated berries were larger than berries from all other treatments in 2001, whereas in 2002 berry weight followed the pattern A > open > AX > (X~0). Sucrose content of juice and speed of ripening were unaffected by treatments.

KEY WORDS pollination, nectar robbing, rabbiteye blueberry, *Vaccinium ashei*, *Apis mellifera*, *Xylocopa virginica*

INTRODUCTION

Carpenter bees (*Xylocopa* Latreille) are common flower visitors in the Southeastern USA (Cane and Payne 1990, Delaplane 1995). Some flowers, including rabbiteye blueberry *Vaccinium ashei* Reade, possess long tubular corollae in which nectar is inaccessible to the carpenter bees, so the

bees obtain nectar by imbibing it through perforations they make with their maxillary galeae in the walls of the calyx or corolla. This behavior is called nectar theft or robbery (Faegri and Van der Pijl 1979, Inouye 1980, 1983). Carpenter bees have been reported to act as nectar thieves in *Petrocoptis grandiflora* (Guitian et al. 1994), ocotillo (*Fouquieria splendens*) (Scott et al. 1993), as well as blueberry (Cane and Payne 1990, Delaplane 1995, Sampson and Cane 2000). Nectar-thieving flower visits often do not result in pollination benefit to the plant (Inouye 1980, 1983, Maloof and Inouye 2000). The removal of floral nectar by robbers decreases the standing crop and in some cases changes the sugar concentration of nectar available to other pollinators (Pleasants 1983).

In the Southeastern USA, *Xylocopa virginica* (L.) (Cane and Payne 1990) and *X. micans* (Lepeletier) (Delaplane 1995) are frequent visitors to commercial blueberry orchards. Both engage in nectar thievery and are considered ineffective pollinators of this native crop species. Delaplane (1995) documented that 100% of blueberry flower visits by both species are acts of nectar thievery. Moreover, the robbery holes in flowers made by *X. virginica* and *X. micans* are attractive to honey bees (*Apis mellifera* L.) – a species commonly imported for commercial pollination. Honey bees are incapable of making robbery holes, but readily visit holes made by *Xylocopa* spp. and thus act as secondary nectar thieves (Delaplane and Mayer 2000). Delaplane (1995) showed that 92.3% of honey bees were observed to rob nectar in the presence of *Xylocopa* spp.

Previous studies have documented the primary nectar thieving activity of carpenter bees and its secondary expression by honey bees, but no information is available about the effect of these behaviors on the pollination efficacy of *A. mellifera* in rabbiteye blueberry. Honey bees are effective pollinators of *V. ashei* var. ‘Climax’ (Sampson and Cane 2000, Dedej and Delaplane

2003), but it is possible that their efficacy is compromised when they are diverted to robbing behavior. Hence, we designed a 2-yr study to compare incidence of honey bee robbing behavior, fruit-set, seed number, berry weight, sucrose content of juice, and speed of ripening in rabbiteye blueberry plants tented with various combinations of *A. mellifera* and *X. virginica*.

MATERIALS AND METHODS

Orchard and experimental setup. The study was conducted at a permanent orchard of the Horticulture Farm of the University of Georgia, Oconee County, GA in 2001 and 2002. Mature rabbiteye blueberry (*V. ashei*) plants in alternating rows of the cultivars 'Climax' and 'Premier' were used. The experiment consisted of caging plants during the bloom period with standardized densities of honey bees and carpenter bees, recording flower visitation behavior of honey bees during bloom, and measuring characteristics of fruit at harvest. Overhead irrigation was used to prevent frost damage to flowers (Eck 1988) when ambient temperatures reached -2°C (1-2 nights per yr). Drought conditions prevailed in 2001-2002, and when farm managers deemed it necessary the whole orchard would be irrigated.

Treatments and bees. Treatments consisted of plots, each with two homogeneous mature 'Climax' plants (15-y old, 1.6–1.8 m) that were either tented with bees or left open as controls. Tents were 1.8 x 1.8 x 1.8 m units covered with Lumite screen (Bioquip Corp., CA, USA). Tented plots were provided two vigorously-flowering 3-gal potted 'Premier' as pollenizers while open plots were not provided potted pollenizers because of the ambient abundance of 'Premier.' We supplemented the potted pollenizers with fresh cuttings of flowering 'Premier' after potted pollenizers exceeded 50% flower drop. Five treatments were organized as follows: (1) honey bees and two carpenter bees combined in one tent (*Apis / Xylocopa*, AX), (2)

two carpenter bees (*Xylocopa*, X), (3) honey bees (*Apis*, A), (4) no bees (0), and (5) open plot (open). Treatments were assigned randomly to tents, and there were two tents per treatment in 2002 and one per treatment in 2001. Because the university research apiary is near the experimental orchard, honey bees, as well as other bee species, were able to visit the open plots freely. Standard densities of 3200 honey bees (initial counts, determined after Delaplane and Hood (1997)) and two carpenter bees per tent were used. The density of 3200 honey bees was chosen because there is evidence that under identical conditions this density is non-limiting in either performance of pollinators or availability of pollenizer pollen (Dedej and Delaplane 2003). The number of two carpenter bees per tent was considered a reasonable compromise between inefficacy (risk of no flower perforations) and satiation (completely perforated); this was shown to be a good choice as the rates of perforated flowers in the AX plots ranged from 0-50% (23.2 ± 2.9, n=60) over the two years of study. Secondly, the effects of *X. virginica* on honey bees are profound at what appear to be low densities; as few as one carpenter bee per 25 bushes, or 4% incidence of punctured corollae can divert 80-90 % of honey bees to robbing behavior (Cane and Payne 1991). And finally, our unpublished observations indicate a natural ambient visitation rate by *X. virginica* per two bushes in this orchard to be 2.3 ± 1.6 (n=19) visits per 2 min.

Honey bees were added to the plots (22 March 2001, 24 March 2002) when advanced buds were at stage 5 (Spiers 1978). Colonies were fed regularly with sugar syrup and socially stabilized with synthetic queen mandibular pheromone (QMP) (one queen equivalent of Bee-Boost™) (Currie et al. 1994). QMP was used in lieu of a queen to eliminate confounding effects of differential brood production resulting from variable bee populations.

Carpenter bees were caught and added to the appropriate tents as soon as they were naturally available. They were checked every day and if missing or dead substituted with fresh

ones, always maintaining two *X. virginica* per tent. Carpenter bees were replaced with fresh ones after being used for one week. We used exclusively male carpenter bees as they are the first active in a season and occur in near-synchrony with the onset of *V. ashei* flowering. The inclusive dates during which *X. virginica* were present in experimental tents were 2 – 23 April 2001 and 28 March – 18 April 2002. Wooden blocks (4 x 8 x 6 cm) each with a 1-cm dia. hole were provided to tents and carpenter bees were occasionally observed to use them as shelters.

Dependent variables. At stages 4-5 of blooming (Spiers 1978) the number of unopened flowers per raceme was determined for 40 tagged racemes distributed *ca*. equally between the two 'Climax" plants in each plot; this was done on 19-20 March 2001 and on 22-23 March 2002 prior to inserting bees into tents. To compensate for wind loss of tags, twice as many racemes (80) were marked in the open plots.

On 14-15 days during the bloom period (2 – 23 April 2001**,** 28 March – 17 April 2002), we measured for each plot (excluding the non-*Apis* tents 0 and X) the proportion of illegitimate honey bee flower visits (based on number of visits per 2 min) during normal flight hours (11:00 – 16:00). Visits during which honey bees probe the terminal aperture of the flower, presumably effecting pollination, were considered legitimate (L) whereas those realized by probing lateral robbery holes made by carpenter bees were considered illegitimate (I) (Faegri and Van der Pijl 1979). The average percentage of honey bee flower visits illegitimate was calculated for A, AX, and open plots as (I ÷ (L + I)) x 100. Data from open plots were subsequently used to make asymptotic models testing various relationships among dependent variables (see below).

To monitor the condition of flowers under ambient orchard conditions, we measured in the open plots on 9, 16, and 23 April 2001 and 4 and 11 April 2002 the percentage of open flowers with lateral perforations in their corollae, calculated as (perforated open flowers ÷

(perforated open + non-perforated open)) x 100. These measurements were made for each of ten racemes per open plot per sampling day (n = 70).

After bloom was finished, all bees were removed and the Lumite screens replaced with poultry netting to protect fruit from animals and unauthorized harvesting.

Percentage fruit-set ([no. fully-formed fruit ÷ no. unopened flowers] x 100) was determined for each recovered raceme while fruit were full-sized but still green. Harvest of ripe fruit was done from 16 June – 15 July 2001 and from 12 June – 15 July 2002. The following dependent variables were measured for each recovered raceme: percentage fruit-set, number of seeds per fruit, berry weight (g), speed of ripening, and sucrose content of juice. Number of seeds per fruit was determined by expressing berry contents and counting the number of apparently viable seeds (Lyrene 1989, MacKenzie 1997, Ritzinger and Lyrene 1998) as discriminated by a single observer. Speed of ripening was calculated as the proportion of fruits ripe per raceme on two arbitrarily chosen dates – 15 June 2001 and 1 June 2002 – when fruit was 30-50% ripe. Fruit juice was collected by piercing each sampled berry with a micro-capillary tube, and percent sucrose content of juice determined with a bench-top refractometer (Fisher, PA, USA). Data for parameters requiring destructive sampling of ripe fruit (number of seeds, berry weight, and sucrose content of juice) were collected sequentially as ripe fruit became available. Fruit weight and sucrose content data were collected within 2 h of harvest; seed counts were sometimes delayed until the next day.

Statistical analyses. The effects of year and treatment on incidence of illegitimate honey bee visits, fruit-set, number of seeds per fruit, berry weight, speed of ripening, and sucrose content of juice were tested with a randomized design analysis of variance (ANOVA) recognizing tent (year x treatment) as error term and testing for interactions of treatment with

year. When treatment and year interacted we analyzed independent variables by year and used residual error as test term. Means were separated by Duncan's test and differences accepted at the $\alpha \leq 0.05$ level (SAS Institute 1992).

The relationships between honey bee robbing behavior (y) and time (x = Julian day) under ambient orchard conditions (open tents) and honey bee robbing behavior (y) and percentage flowers perforated (x) were described using the Mitscherlich asymptotic growth model (Ware et al. 1982) and the NLIN procedure (SAS Institute 1992). The model was $y = \beta(1 - pe^{-\alpha(x - x_p)})$, where β = maximum asymptotic value of y as x approaches ∞, e = exponential function, α = constant of proportionality, and x_p = the value of x where y reaches 1- p of β. In our case β was restricted to ≤ 100, and p was set to 0.05, which solved the value of x_p with 0.95 of β.

RESULTS AND DISCUSSION

Honey bee foraging behavior on *V. ashei* and condition of flowers. The percentage of honey bee flower visits illegitimate in open, A, and AX plots was affected by treatment (F=1048.3; df=2,3; P<0.0001); there were no year effects or interactions of treatment with year ($F \leq 4.8$; df=1 or 2,3; $P \geq 0.1153$). The mean separations in Table 4.1 indicate that differences were wholly explained by the co-occurrence of honey bees with *X. virginica*. Nectar thievery by honey bees was highest in open plots followed by AX tents, but non-existent in the A tents from which *X. virginica* were excluded. These data confirm the high incidence of secondary nectar thievery by honey bees in Southeastern blueberry plantations (Delaplane 1995) as well as the inability of honey bees to act as primary nectar thieves. Nectar thievery by honey bees is dependent on the primary thieving activity of *Xylocopa* spp. during which the corollae receive the perforations that are subsequently preferred by honey bees.

Under ambient orchard conditions, temporal changes in the incidence of honey bee robbing behavior were explained by Mitscherlich asymptotic growth models (Fig. 4.1) in which rates of illegitimate honey bee flower visitation increase from initial lows to fixation at ≥95%. For 2001, the model predicted that the incidence of honey bee robbing behavior would achieve 95% by Julian day 97 (x_p, out of a range of observed days Julian 92–113). The same value for 2002 was Julian day 90 (range of observed days Julian 87–107). We propose two explanations for the temporal increase in robbing behavior by honey bees: increasing availability of flowers perforated by *X. virginica* and/or learned preferential transition by honey bees from legitimate to illegitimate foraging. The first explanation is only weakly supported by the data. Once *X. virginica* were strongly active in the orchard (>22 March 2001, >1 April 2002), the average incidence of flowers perforated in the open plots generally increased over the three consecutive weekly sampling periods beginning 1-2 April (30.6 ± 4.5, 30.2 ± 4.6, and 42.9 ± 7.1% for weeks 1-3, respectively), but not significantly (F=2.8; df=2,1; P>0.4). Instead, the average range of 30.2 – 42.9% open flowers perforated was achieved early then remained static. Therefore, honey bees do not appear to be responding to temporal variation in the availability of perforated flowers. However, when the relationship between proportion of honey bee visits illegitimate and proportion of flowers perforated was examined independently of time, almost all the variation (r^2 = 0.99) was explained by a Mitscherlich asymptotic growth model (Fig. 4.2) which predicts that illegitimate foraging by honey bees increases from initial lows to fixation at ≥95% once the incidence of perforated flowers achieves 50% (x_p). Collectively these analyses suggest that the incidence of perforated flowers in an orchard rapidly achieves static levels but that honey bees learn quickly to preferentially visit flowers illegitimately, reaching near-fixation in the expression of this behavior. Indeed, we note a type of flower rejection behavior in honey bees by

which the forager inspects the proximal base of corollae and rejects those without perforations. In one sampling interval in 2001, these rejections constituted 21.9 ± 10.3 (n=3) of honey bee flower visits in the AX plots. Honey bees are known to express similar learned transitions in flower handling behavior in other crop species, most notably alfalfa (*Medicago sativa* L.) in which the bees learn to avoid tripping the sexual column, thus avoiding a blow to the head (Tucker 1956).

Fruit-set. Fruit-set was affected by treatment (F=13.6; df=4,5; P=0.0068), but there were no year effects or interactions of treatment with year ($F \leq 4.0$; df=1 or 4,5; $P \geq 0.0822$). Fruit-set was significantly higher in the A, AX, and open plots than X or 0 plots (Table 4.1). These results support earlier work showing that *A. mellifera* is an effective pollinator of rabbiteye blueberry var. 'Climax' (Sampson and Cane 2000; Chapter 3 in this thesis). The similarity in fruit-set between A and AX plots is noteworthy considering that the incidence of illegitimate flower visits by honey bees approached 40% in the AX plots (Table 4.1). Moreover, commercially-acceptable yields are produced in orchards in which the incidence of illegitimate honey bee visitation meets or exceeds 95% (Fig. 4.1). Even though bees were excluded from the 0 plots, an average background of 25.1% fruit-set (selfing) is implicated with *V. ashei* var. 'Climax' in this orchard (Table 4.1). Although *V. ashei* is considered self-sterile, there is a degree of variation in the expression of this character (Meader and Darrow 1944).

Five derivative hypotheses follow from these observations: (1) fruit-set is not compromised by rates of illegitimate honey bee visitation approaching 40%, (2) repeat flower visits by colonial *A. mellifera* effect a degree of compensation for illegitimate visits, given that illegitimate flower visitors are generally inefficient pollinators (Inouye 1980) and that *V. ashei* 'Climax' responds positively to repeat legitimate honey bee visits (Dedej and Delaplane 2003),

(3) a disproportionately high amount of honey bee-induced fruit-set occurs before the bees learn to seek robbery perforations (naïve honey bees are comparatively efficient pollinators [Delaplane and Mayer 2000]), (4) honey bees effect acceptable pollination even at extremely low rates of legitimate flower visitation (unsupported by Dedej and Delaplane [2003]), and/or (5) there is a high degree of compensatory pollination by other legitimate flower visitors, namely *Bombus* spp. Latreille and *Habropoda laboriosa* (F.) (Cane and Payne 1990, see also Stout et al. 2000).

Seed Number. For this variable there were no year effects (F=1.5; df=1,5; P=0.2785), but there was a significant interaction between treatment and year (F=6.0; df=4,5; P=0.0378) so seed number was analyzed by year. There were significant treatment effects in 2001 (F=46.5; df=4,138; P<0.0001) and 2002 (F=123.8; df=4,281; P<0.0001). Mean separations are shown in Table 4.2. The interaction is explained by the inversion of rankings between the open and A plots in the two years.

The number of seeds is a good indicator of the effectiveness of the pollinator as well as a measure of female fertility if compatible pollen is abundant (Ritzinger and Lyrene 1998). Fruits had similarly low number of seeds in plots with no bees or only *X. virginica*, whereas the highest seed numbers were achieved in open or A plots (Table 4.2). The most interesting observation is the comparison of seed numbers between the A and AX plots. In both years there was a reduction of seeds when *A. mellifera* was tented with *X. virginica* (AX plots). This, coupled with the fact that illegitimate flower visitation by honey bees was nearly 40% in the AX plots (Table 4.1), indicates that *X. virginica*-induced nectar robbery by *A. mellifera* compromises the pollination efficacy of *A. mellifera* in *V. ashei* as measured in seed-set. If seed-set continues to drop at increasing rates of secondary nectar thievery, this may have negative effects on fruit-set under ambient conditions where rates of illegitimate honey bee visitation meet or exceed 95%

(Fig. 4.1). Some of our observations support this idea. In three years of field studies (including the present work) we have noted evidence that our orchard is naturally pollinated at levels below its physiological capacity. Fruit-set tends to be lower in open plots than in tented plots in which honey bee densities are optimized (Table 4.1 and Table 3.1 in this thesis). It is likely that *X. virginica*-induced robbing behavior by *A. mellifera* contributes to this ambient pollination deficit. This effect may be exaggerated in commercial plantations of *V. ashei* where imported honey bees are the most numerous bee visitor (Delaplane 1995) and their diversion more damaging. It may be possible to improve the efficacy of honey bees by increasing hive densities and/or moving hives into the orchard after bloom has commenced – practices believed, respectively, to increase the overall number of honey bee foragers and the number of naïve foragers (Delaplane and Mayer 2000). Alternatively, increasing the density of non-*Apis* pollinators is an appropriate response, but these bees are comparatively difficult to manage (Delaplane and Mayer 2000). Another approach is to develop practices that minimize the impact of primary nectar thievery by *Xylocopa* spp.

In general the effects of nectar robbers are a result of complex interactions between the taxa of plants as well as those of legitimate and illegitimate flower visitors (Maloof and Inouye 2000). Indeed, the effects of robbers can range from benign to beneficial, as shown by Maloof (2001) who not only failed to detect a negative effect of nectar thievery on fruit-set and seed-set in *Corydalis caseana*, but suggested that the activity of robbers induces longer inter-floral flight by legitimate visitors thus increasing rates of out-crossing.

Finally, we note that fruit-set remains high in AX plots in spite of a significant decrease in seed-set by *A. mellifera* in the presence of *X. virginica* (Tables 4.1 and 4.2). To the five derivative hypotheses we offer above we now add a tentative sixth: (6) there is at least a partial

physiologic independence of fruit-set from seed-set in *V. ashei*. Support for this hypothesis is found in our earlier independent work (Dedej and Delaplane 2003), but seed-set and fruit-set vary positively in the data of Gupton and Spiers (1994) for rabbiteye and MacKenzie (1997) for highbush cultivars.

Berry Weight. This variable expressed a significant year effect (F=12.8; df=1,5; P=0.016) as well as an interaction between treatment and year (F=5.4; df=4,5; P=0.0466), so it was analyzed by year. There were significant treatment effects in 2001 (F=8.0; df=4,140; P<0.0001) and 2002 (F=32.9; df=4,286; P<0.0001). Mean separations are shown in Table 4.2. Average berry weight (g) was higher in 2002 (1.3 ± 0.02, x ± SE, n=291) than in 2001 (1.0 ± 0.04, n=145). The interaction of treatment with year (Table 4.2) is best explained by the comparatively low sample sizes of 2001 combined with an inversion of rankings between the two years for the open and A plots. However, for 2002 there is evidence that berry weight was higher in open, A, and AX plots over that in 0 or X plots. It is possible that berry weight in *V. ashei* does not respond consistently to variation in pollinator efficiency; Dedej and Delaplane (2003) failed to detect differences in berry weight across a continuum of honey bee densities. However, it is generally expected in *Vaccinium* spp. that berry weight will vary positively with seed number (Filmer and Marucci 1963, Dorr and Martin 1966, Brewer and Dobson 1969, Moore et al. 1972) but this trend is not universal, as demonstrated by MacKenzie (1997) in highbush blueberry.

Sucrose Content. None of the independent variables significantly affected the percent sucrose content of fruit juice (F≤5.0; df=1 or 4,5; P≥0.0767). Our mean value range of 13.7 – 16.0% is generally higher than that of *ca*. 10 – 13.5% reported by Austin and Bondari (1993) for 'Climax.' There was a trend in the data toward higher sucrose content in those plots experiencing

the poorest pollination (Table 4.1), a tendency noted before (Chapter 3 in this study). Cross-pollination is known to increase total sugar content in highbush blueberry (Kobashi et al. 2002).

Speed of Ripening. Speed of fruit ripening was unaffected by any of the independent variables ($F \leq 3.9$; df=1 or 4,5; $P \geq 0.1038$). Excepting the AX plot, there was a trend in the data for faster ripening in those plots experiencing superior pollination (Table 4.1). Cross-pollination with compatible pollen is known to improve speed of ripening in highbush (MacKenzie 1997) and rabbiteye blueberries (Gupton and Spiers 1994).

Conclusion. Our data confirm a high incidence of primary nectar thievery by *X. virginica* in Southeastern *V. ashei* plantations, the inability of honey bees to engage in primary thievery, a high incidence of secondary thievery by honey bees, and implicate a learned preference by honey bees for corollae formerly perforated by *X. virginica*. Under tent conditions the continuous presence of 2 carpenter bees per 2 mature bushes results in nearly 40% incidence of *A. mellifera* robbery. This level of secondary nectar thievery does not reduce fruit-set but does reduce the number of seeds per berry – an important physiological indicator of pollinator efficacy. There appears to be a partial independence of fruit-set from seed-set in *V. ashei* (fruit-set is optimal [Table 4.1] in plots in which seeds / berry is sub-optimal [Table 4.2]). But if seed-set is further reduced under ambient conditions where the incidence of secondary thievery meets or exceeds 95% (Fig. 4.1), then it is possible that secondary thievery eventually exacts a cost on fruit-set. This scenario may explain the sub-optimal fruit-set we have observed under ambient orchard conditions.

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REFERENCES

Austin, M.E. and K. Bondari. 1993. Fruit quality characteristics of five rabbiteye blueberry cultivars and clones. Acta Hortic. 345: 133-140.

Brewer, J.W. and R.C. Dobson. 1969. Seed count and berry size in relation to pollinator level and harvest date for the highbush blueberry, *Vaccinium corymbosum*. J. Econ. Entomol. 62(6): 1353-1356.

Cane, J.H. and J.A. Payne. 1990. Native bee pollinates rabbiteye blueberry. Alabama Agric. Exp. Sta. 37(4): 4.

Cane, J.H. and J.A. Payne. 1991. Native bee pollinates rabbiteye blueberry. Alabama. In: Proceedings of the Southeast Blueberry Conference, Tifton , Georgia, USA.

Currie, R.W., M.L. Winston and N.K. Slessor. 1994. The effect of honey bee (*Apis mellifera* L.) synthetic queen mandibular compound on queenless 'Disposable' pollination units. Am. Bee J. 134: 200-202.

Delaplane, K. S. 1995. Bee foragers and their pollen loads in South Georgia rabbiteye blueberry. Am. Bee J. 135: 825-826.

Delaplane, K.S. and W.M. Hood. 1997. Effects of delayed acaricide treatment in honey bee colonies parasitized by *Varroa jacobsoni* and a late-season treatment threshold for the south-eastern USA. J. Apic. Res. 36(3/4): 125-132.

Delaplane, K.S. and D.F. Mayer. 2000. Crop pollination by bees. CABI Publishing, Oxon, UK, 344 pp.

Dorr, J. E. and E. C. Martin. 1966. Pollination studies on the highbush blueberry, *Vaccinium corymbosum* L. Mich. Agric. Exp. Sta. Quart. Bull. 48: 437-448.

Eck, P. 1988. Blueberry science. Rutgers University Press, New Brunswick, NJ, USA.

Faegri, K. and L.W. Van der Pijl. 1979. The principles of pollination ecology (third edition). Pergamon, Oxford, UK, 244 pp.

Filmer, R.S. and P.E. Marucci. 1963. The importance of honeybees in blueberry pollination. 31st Ann. Blueberry Open House Proc., North Carolina Agric. Exp. Sta., pp. 14-21.

Gordon, W.F., B. S. Vinson and A. Lewis. 1979. Territorial behavior in male *Xylocopa micans* (Hymenoptera: Anthophoridae). J. Kansas Entomol. Soc. 52(2): 313-323.

Guitian, J., J.M. Sanchez and P. Guitian. 1994. Pollination ecology of *Petrocoptis grandiflora* Rothm. (Caryophyllaceae); a species endemic to the north-west part of the Iberian Peninsula. Bot. J. Linnaean Soc. 115(1): 19-27.

Gupton, C.L. and J.M. Spiers. 1994. Interspecific and intraspecific pollination effects in rabbiteye and southern highbush blueberry. HortScience 29(4): 324-326.

Inouye, D.W. 1980. The terminology of floral larceny. Ecology 61: 1251-1253.

Inouye, D.W. 1983. The ecology of nectar robbing. *In* (B. Bentley and T. Elias, eds.) The biology of nectaries. Columbia University Press, New York, New York, USA, pp. 153-173.

Kobashi, K., S. Sugaya, M. Fukushima, and S. Iwahori. 2002. Sugar accumulation in highbush blueberry fruit as affected by artificial pollination with different pollen sources in relation to seed number, invertase activities and ABA content. Acta Hortic. 574: 47-51.

Lyrene, P.M. 1989. Pollen source influences fruiting of 'Sharpblue' blueberry. J. Am. Soc. Hortic. Sci. 114(6): 995-999.

MacKenzie, K.E. 1997. Pollination requirements of three highbush blueberry (*Vaccinium corymbosum* L.) cultivars. J. Am. Soc. Hortic. Sci. 122(6): 891-896.

Maloof, J.E. 2001. The effects of a bumble bee nectar robber on plant reproductive success and pollinator behavior. Am. J. Bot. 88: 1960-1965.

Maloof, J.E. and D.W. Inouye. 2000. Are nectar robbers cheaters or mutualists? Ecology 81: 2651-2661.

Meader, E.M. and G.M. Darrow. 1944. Pollination of the rabbiteye blueberry (*Vaccinium ashei*) and related species. Proc. Am. Soc. Hortic. Sci. 45: 267-274.

Moore, J.N., B.D. Reynolds, and G.R. Brown. 1972. Effects of seed number, size, and development on fruit size of cultivated blueberries. HortScience 7: 268-269.

Pleasants, J.M. 1983. Nectar production in *Ipomopsis aggregata* (Polemoniaceae). Am. J. Bot. 70: 1468-1475.

Ritzinger, R. and P.M. Lyrene. 1998. Comparison of seed number and mass of southern highbush blueberries vs. those of their F_1 hybrids with *V. simulatum* after open pollination. HortScience 33(5): 887-888.

Sampson, B. and J. Cane. 2000. Pollination efficiencies of three bee (Hymenoptera: Apoidea) species visiting rabbiteye blueberry. J. Econ. Entomol. 93: 1726-1731.

SAS Institute 1992. SAS/STAT user's guide, version 6. SAS Institute; Cary, NC, USA, 846 pp. (4th edition).

Scott, P.E., S.L. Buchmann and M.K. Orourke. 1993. Evidence for mutualism between a flower piercing carpenter bee and ocotillo – use of pollen and nectar by nesting bees. Ecol. Entomol. 8(3): 234-240.

Spiers, J.M. 1978. Effect of stage of bud development on cold injury in rabbiteye blueberry. J. Am. Soc. Hortic. Sci. 103: 452-455.

Stout, J.C., Allen, J.A. and D. Goulson. 2000. Nectar robbing, forager efficiency and seed set: Bumblebees foraging on the self incompatible plant *Linaria vulgaris* (Scrophulariaceae). Acta Oecol. 21(4-5): 277-283.

Tucker, K.W. 1956. Honey bee pollinators of alfalfa in Minnesota. Rep. 15th Alfalfa Improvement Conf. pp. 32-33.

Ware, G.O., Ohki, K. and L.C. Moon. 1982. The Mitscherlich plant growth model for determining critical nutrient deficiency levels. Agron. J. 74: 88-91.

Table 4.1. Rates of illegitimate honey bee visitation and fruit characteristics (% fruit-set, sucrose content and speed of ripening) of 'Climax' rabbiteye blueberry as affected by honey bees and carpenter bees in tents (*ca*. 2 x 2 m). Five treatments were organized as follows: 3200 honey bees and two carpenter bees combined in one tent (*Apis* / *Xylocopa*, AX), two carpenter bees (*Xylocopa*, X), honey bees (*Apis*, A), no bees (0), and open plot (open). Data are pooled for years 2001 and 2002. Values are mean ± standard error, with *n* in parentheses. Means within a column followed by the same letter are not significantly different at the $\alpha = 0.05$ level.

Treatment	Illegitimate *A. mellifera* visits (%)	Fruit-set (%)	Sucrose content of juice (%)	Speed of ripening (%)
Open	90.1 ± 3.5 (38)a	59.9 ± 2.4 (198)a	13.7 ± 0.24 (112)a	46.8 ± 2.8 (158)a
A	0 (36)c	71.3 ± 2.6 (115)a	13.9 ± 0.3 (103)a	41.2 ± 3.3 (90)a
AX	39.4 ± 6.0 (36)b	73.6 ± 2.2 (113)a	13.8 ± 0.3 (97)a	19.8 ± 2.5 (110)a
X	NA	21.9 ± 2.3 (110)b	15.5 ± 0.3 (63)a	23.4 ± 4.0 (64)a
0	NA	25.1 ± 2.7 (110)b	16.0 ± 0.3 (56)a	26.1 ± 4.6 (64)a

Table 4.2. Fruit characteristics (number of mature seeds per berry and weight of berry) of 'Climax' rabbiteye blueberry as affected by honey bees and carpenter bees in tents (*ca*. 2 x 2 m). Five treatments were organized as follows: 3200 honey bees and two carpenter bees combined in one tent (*Apis* / *Xylocopa*, AX), two carpenter bees (*Xylocopa*, X), honey bees (*Apis*, A), no bees (0), and open plot (open). Treatment interacted with year for seed number and berry weight; hence data are here reported by year (2001 and 2002). Values are mean ± standard error, with *n* in parentheses. Means within a column followed by the same letter are not different ($\alpha = 0.05$ level).

Treatment	**Mature seeds / berry**		**Berry weight (g)**	
Year	**2001**	**2002**	**2001**	**2002**
Open	14.6 ± 1.4 (25)b	24.4 ± 1.0 (86)a	1.4 ± 0.1 (25)a	1.5 ± 0.04 (88)b
A	18.6 ± 1.3 (38)a	17.0 ± 1.0 (63)b	0.8 ± 0.05 (38)b	1.6 ± 0.05 (65)a
AX	10.3 ± 0.7 (37)c	10.6 ± 0.7 (64)c	1.07 ± 0.06 (37)b	1.2 ± 0.04 (65)c
X	3.1 ± 0.6 (26)d	0.7 ± 0.3 (35)d	0.9 ± 0.07 (28)b	0.9 ± 0.06 (34)d
0	0.5 ± 0.2 (17)d	0.08 ± 0.06 (38)d	0.8 ± 0.09 (17)b	1.0 ± 0.05 (39)d

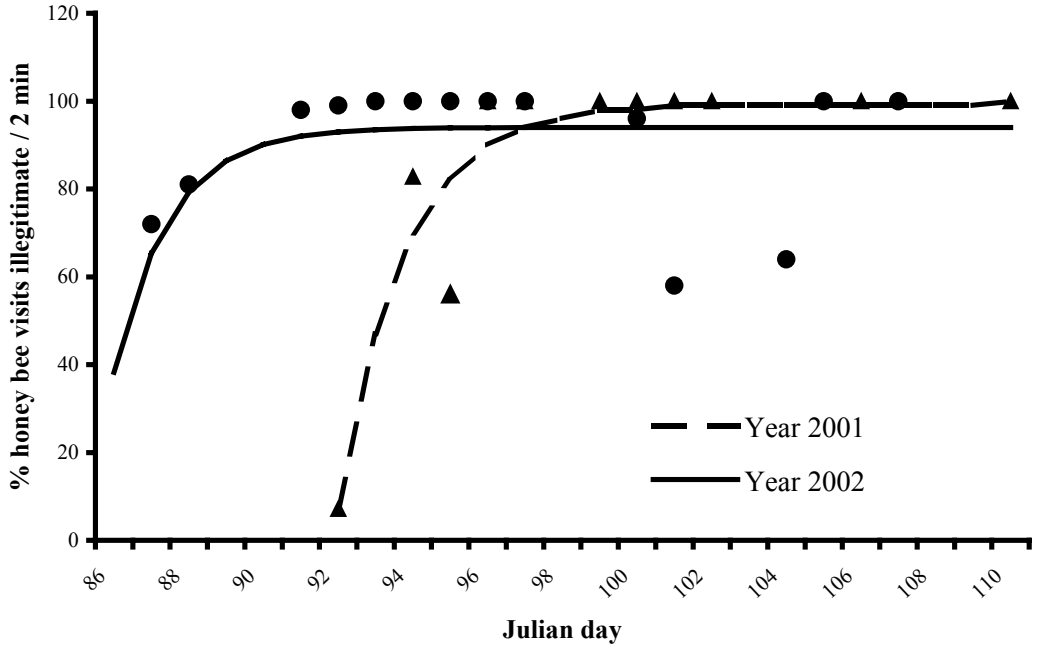

Fig. 4.1. Relationship between Julian day (x) and percentage of honey bee flower visits illegitimate / 2 min (y) in open plots of 'Climax' rabbiteye blueberry. Observed values (2001 ▲; 2002 ●) are percentage of honey bee visit illegitimate, and the lines connect predicted values from Mitscherlich asymptotic growth models (Ware et al. 1982): $y_{2001}=100(1–0.05e^{-0.56(x\,-\,97.2)})$, $r^2 = 0.88$; $y_{2002}=100(1–0.05e^{-0.67(x\,-\,89.7)})$, $r^2 = 0.86$.

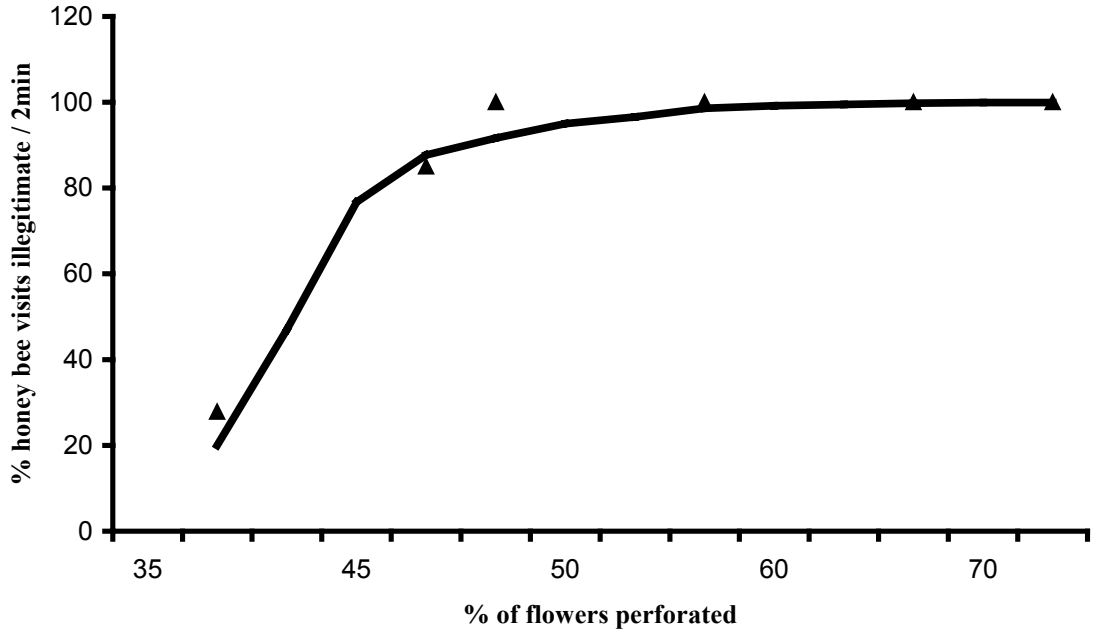

Fig. 4.2. Relationship between percentage of flowers perforated (x) and percentage of honey bee flower visits illegitimate / 2 min (y) in open plots of 'Climax' rabbiteye blueberry. Observed values (▲) are percentage of honey bee visits illegitimate and the line connects predicted values from the Mitscherlich asymptotic growth model (Ware et al. 1982): $y=100(1–0.05e^{-0.19(x\ -\ 50.0)})$, $r^2 = 0.99$.

CHAPTER 5

NET ENERGY ADVANTAGE DRIVES HONEY BEES (*APIS MELLIFERA* L.) TO NECTAR LARCENY IN *VACCINIUM ASHEI* READE[1]

[1] Dedej S. and K. S. Delaplane. Submitted to *Behavioral Ecology and Sociobiology*, 3/30/2004.

ABSTRACT

Carpenter bees (*Xylocopa* spp.) act as primary nectar thieves in rabbiteye blueberry (*Vaccinium ashei* Reade), piercing corollae laterally to imbide nectar at basal nectaries. Honey bees (*Apis mellifera* L.), learn to visit these perforations and thus become secondary nectar thieves. We tested the hypothesis that honey bees make this behavioral switch in response to an energetic advantage realized by illegitimate flower visits.

Nectar volume and sugar quantity were higher in intact than perforated flowers on every sampling day, and this pattern was the same in flowers before or after one bee visit. Percentage of available nectar and sugar removed by one bee visit were higher in perforated than intact flowers. The number of *V. ashei* flowers visited per min, nectar ingestion rate, and net energy gain per sec handling time were either unaffected by type of bee visit or higher for illegitimate than legitimate *A. mellifera* flower visitors. Total foraging time per flower and foraging energy spent per flower were either unaffected by type of visit or higher for legitimate flower visitors.

There were significant effects of type of bee visit for net energy gain per flower on every sampling day; however, the trend was inconsistent with higher net energy gain for legitimate visitors on four of six days and the reverse pattern the other two. On average, honey bees spent more time as well as energy handling one flower during legitimate visits than during robbing. We conclude that the majority evidence indicates an energetic advantage for honey bees that engage in secondary nectar thievery in *V. ashei*.

KEY WORDS Honey bees, *Apis mellifera*, carpenter bees, *Xylocopa virginica*, rabbiteye blueberry, *Vaccinium ashei*, nectar larceny, foraging, Apoidea

INTRODUCTION

Some flowers have morphological features that impede access by certain taxa of nectarivores, including bees. One example of this is rabbiteye blueberry *Vaccinium ashei* Reade, a bush-type species native to the southeastern USA where it is cultured commercially. The *V. ashei* flower has a long tubular corolla with basal nectaries relatively inaccessible to those short-tongued bees ordinarily restricted to gaining access at the aperture. In the case of *V. ashei* in the southeastern USA, however, there is an interesting syndrome of primary and secondary nectar larceny. The native short-tongued carpenter bee, *Xylocopa virginica* L., gains access to *V. ashei* nectar by imbibing it through perforations it makes with its maxillary galeae in the lateral walls of the corolla, an example of primary nectar larceny (Faegri and Van der Pijl 1979, Inouye 1980, 1983). The exotic, short-tongued honey bee, *Apis mellifera* L., subsequently visits these perforations and becomes a secondary thief (Delaplane and Mayer 2000). The effects of nectar larceny on plant reproduction are variable. Even though the removal of floral nectar decreases the standing crop and changes sugar concentration of nectar available to other pollinators (Pleasants 1983), its effects on plant reproduction range from benign (Maloof 2001) to damaging (Inouye 1980, 1983, Maloof and Inouye 2000, Dedej and Delaplane 2004).

In the local syndrome described above, *A. mellifera* is incapable of perforating the *V. ashei* corolla and learns to transition from aperture visits (legitimate) to illegitimate lateral visits (larceny) only after *X. virginica* becomes active in the orchard. Dedej and Delaplane (2004) showed that this transition from legitimate to illegitimate flower visitation is rapid, going from initial lows to near-fixation at 95% within 4-6 days of observation (of 21-22 observed). The most plausible explanation for this rapid behavioral transition is an energetic advantage for illegitimate flower visits over legitimate. This study was designed to test this hypothesis.

MATERIALS AND METHODS

General. The study was conducted during spring 2003 at a mature 15-y old of *V. ashei* at the Horticulture Farm of the University of Georgia, Oconee County, GA, USA.

Nectar was extracted without excising flowers using calibrated 1-5 µl disposable pipettes (Fischer Scientific, Pittsburgh, PA, USA) (Corbet 2003); nectar volume was measured to the nearest 0.1 µl. Sugar concentration was measured as % Brix (g sucrose per 100 g solution) (Corbet 2003) using a hand-held refractometer model MT-098 (Three-In-One Ent. Co., LTD., Taipei, Taiwan) with range 0-80 % Brix and accuracy to the nearest 0.5%. The Brix percentage was temperature-corrected using the correction table provided by manufacturer. The quantity of sucrose per sample (mg) was calculated by multiplying volume of nectar x temperature-corrected % Brix x the sucrose density values after Dafni (1992, p. 148).

Nectar standing crop in intact and *X. virginica*-perforated flowers and quantity of nectar ingested by honey bees per *V. ashei* flower visit. With the intention to calculate how much energy honey bees gain during legitimate or illegitimate flower visits, we measured nectar standing crop (µl) in unvisited flowers (before bee visitation on that day) (n=299) and in flowers after one visit by a honey bee (n=275) on six days (28 and 29 March and 1, 2, 4, and 14 April). These measures were taken for both perforated and intact flowers. Nectar standing crop in unvisited flowers was measured early in the morning before bees started visiting flowers (28 and 29 March) or later using cages (1.8 x 1.8 x 1.8 m frames covered with Lumite screen (Bioquip, CA, USA)) to exclude large insects. After measuring nectar standing crop in unvisited flowers (in both perforated and intact flowers), the screen of the cage was removed and honey bees allowed to visit flowers freely. Once a flower was observed to be visited by a honey bee, the flower was immediately excised and placed in a plastic container. After collecting 20-25 flowers

(perforated and intact) for that day, we measured their nectar standing crop; this represented average nectar standing crop remaining in flowers after one bee visit. Daily average quantity of sugar (mg) **(S)** or volume (µl) ingested by one honey bee from one flower visited was calculated as the difference between the quantity of nectar standing crop before and after bee visitation. We were also able to calculate percentage of available nectar (µl), percentage of available sugar (mg), as well as absolute amount of sugar (mg) removed by one bee visit. Nectar ingestion rates for both legitimate and illegitimate foragers were calculated as the average volume (µl) of nectar per flower for a particular day divided by handling time (sec, see **H** below).

Honey Bee Observations and Foraging Energetics. Foraging in insects involves four components that have unique thermoregulatory requirements and constraints (Heinrich 1993): warm-up prior to take off, intermittent flight between flowers, perching or walking on flowers, and continuous flight to and from the nest. As the first and fourth responses in our study were assumed the same for both legitimate and illegitimate foragers (experimental orchard and apiary are next to each other), we focused only on the intermittent flight between flowers and perching or walking on flowers.

The intermittent flight time between flowers (Heinrich 1993) or inter-flower movement (Seeley 1985) spent by honey bees approaching (either hovering or not) and landing at flowers was defined as the discrimination time **(D)** as honey bees spend this time to discriminate between inflorescences in plants (Gilbert et al. 1991), while the time spent probing floral apertures or, in our case lateral robbery holes, was defined as handling time **(H)** (Seeley 1985, Dafni 1992). We assume (after Gilbert et al. 1991) that handling time corresponds to ingestion time. Handling time is well defined and used in the literature (Seeley 1985, Gilbert et al. 1991, Dafni 1992,

Kearns and Inouye 1993). For our purposes we define total observation time as (Discrimination + Handling time), noted as **(T)**.

Over the six days of sampling for nectar standing crop (see above) we monitored and timed (with a hand-held stopwatch) the foraging activity of 102 honey bees on *V. ashei* flowers. These observations were made as soon as possible after measuring standing crop. On each day, 10 - 20 bees performing different types of visitation (legitimate or robbing) were each opportunistically noticed (sec = 0) and followed until they were lost to sight (Stout et al. 2000). For each bee, total time of observation **(T)**, time spent discriminating **(D)**, time spent probing and ingesting **(H)** as well as number of flowers visited and type of visit performed were recorded. Individual honey bees displaying mixed behaviors (legitimate + illegitimate, legitimate + rejection, or illegitimate + rejection) were excluded from analysis as their number was low (*n*=3). Rejection is indicated when a forager inspects the proximal base of corollae and rejects those without perforations.

Two stopwatches (Kearns and Inouye 1993) were used to monitor honey bee foraging behavior. With one stopwatch the total time of observation **(T)** for an individual bee was recorded, while with the other the handling time **(H)** of the same bee was recorded for every flower visited as long as the bee was observed. The second stopwatch was stopped every time the bee stopped probing one flower and was restarted when it began probing another. **(D)** was derived as the difference between **(T)** and **(H)**.

The net energy gain (J) was calculated for both legitimate and illegitimate honey bee foragers using the following equation modified from Harder and Cruzan (1990):

$$\mathbf{N_E = I_E - O_E}$$

where $\mathbf{N_E}$ = Net energy gain, $\mathbf{I_E}$ = Intake energy and $\mathbf{O_E}$ = Output energy (energy spent for flight activity during discrimination and handling).

Intake energy (J) was calculated as

$$\mathbf{I_E = nSe}$$

where **n** = number of flowers visited during total observation time **(T), S** = average quantity of sugar ingested from one flower visited (mg) (see above), and **e** = energy content (J) of 1 mg sugar (1 mg sugar is equivalent to 4.2 calories or 17.6 J (4.2 x 4.186 = 17.6 J)) (Schmidt-Nielsen 1997).

The output energy (J) was calculated as

$$\mathbf{O_E = D_E + H_E}$$

where $\mathbf{D_E}$ = quantity of energy spent by honey bee during discrimination time and $\mathbf{H_E}$ = energy spent during handling time. These were derived from

$$\mathbf{D_E = wt_d\, k_d}$$

and

$$\mathbf{H_E = wt_h\, k_h}$$

where **w =** bee mass (125 mg), $\mathbf{t_d}$ = total time spent during intermittent flights to inflorescences (sec) (discrimination time), $\mathbf{t_h}$ = total time (sec) spent handling flowers during observations and $\mathbf{k_d}$ and $\mathbf{k_h}$ = honey bee's mass-specific rates of energy expenditure during inter-floral flight and handling respectively (J/g/sec). $\mathbf{k_d}$ and $\mathbf{k_h}$ were derived from published values of honey bee oxygen consumption. Oxygen consumption during discrimination time was calculated as an average of values reported by Wolf et al. (1989) for empty and loaded bees during free directional flight (velocity 0.5 m/sec) equal to 91.65 ml O_2/g/h. To calculate energy expended in J, the volume of oxygen consumed was multiplied by 21.3 (1 ml oxygen consumption for honey

bees is equivalent to 21.3 J (Harrison et al. (2001)), giving us a value of 1,952.1 J/g/h or 0.542 J/g/sec. For handling, we again referred to the value reported by Wolf et al. (1989) for walking and motionless bees (11.8 ml O_2/h/bee) which on a bee mass basis (125 mg) is 94.4 ml O_2/g/h, which when multiplied by 21.3 J gives us a value of 2010.7 J/g/h or 0.559 J/g/sec. Using these values and average bee mass of 125 mg, we determined the rate of energy expenditure to be 0.0678 J/bee/sec for discrimination ($\mathbf{k}_d$) and 0.0698 J/bee/sec for handling ($\mathbf{k}_h$). Feurbacher et al. (2003) recently confirmed the rates for loaded nectar foragers reported by Wolf et al. (1989).

Statistical Analyses. The effects of flower type (intact or perforated) and Julian day on nectar volume (µl), sugar quantity (mg), and sugar concentration (%) were analyzed before and after one bee visit with a completely randomized design analysis of variance (ANOVA) recognizing the interaction of day x flower type as test term. An interaction was detected for all variables ($F \geq 5.1$; df≤2,175; $P \leq 0.002$), so each was analyzed by day using residual error as test term (Table 5.1). The effects of flower type (intact, perforated) on percentage of available nectar (µl), percentage of available sugar (mg), and absolute amount of sugar (mg) removed by one bee visit were analyzed with analysis of variance recognizing residual error as test term (Table 5.2). Effects of type of flower visit performed by honey bees (legitimate or illegitimate) on time spent probing and ingesting per flower**,** energy spent per bee handling one flower (Table 5.3), number flowers visited per min, total foraging time per flower, foraging energy spent per flower, net energy gain per flower, net energy gain per sec handling, and nectar ingestion rate (Table 5.4) were tested with a completely randomized ANOVA blocked on Julian days and recognizing the interaction of day x type of visit as test term. When type of visit and day interacted we analyzed independent variables by day and used residual error as test term (Table 5.4). Means were separated by Duncan's test, and differences accepted at the $\alpha \leq 0.05$ level (SAS Institute 1992).

RESULTS

Nectar standing crop in intact and *X. virginica*-perforated flowers and quantity of nectar ingested by honey bees per *V. ashei* flower visit. Nectar volume was higher in intact than perforated flowers on every sampling day, and this pattern was the same in flowers before or after one bee visit ($F \geq 6.7$; df=1,≥29; $P=0.0001$) (Table 5.1). This pattern was the same for sugar quantity ($F \geq 10.2$; df=1,≥29; $P \leq 0.0025$) (Table 5.1), but for sugar concentration there was no interpretable trend with highest values switching repeatedly between intact or perforated flowers by day (Table 5.1). Flower type (intact, perforated) affected the percentage of available nectar and sugar removed by one bee visit ($F \geq 11.5$; df=1,10; $P \leq 0.0069$), but not absolute amount of sugar removed (Table 5.2).

Honey Bee Observations and Foraging Energetics. In this section we present first those results concerning honey bee foraging observations, then report results on foraging energetics. Mean values for variables are distributed between Tables 5.3 and 5.4 depending on the presence or absence of ANOVA interactions in their respective analyses.

The number of *V. ashei* flowers visited per min was either unaffected by type of bee visit or (on 4 of 6 sampling days) higher by illegitimate than legitimate *A. mellifera* flower visitors ($F \geq 5.5$; df=1,≥10; $P \leq 0.0309$) (Table 5.4). Total foraging time per flower **(T)** (Discrimination + Handling time) was either unaffected by type of visit or (on 3 of 6 sampling days) longer for legitimate flower visitors ($F \geq 4.6$; df=1,≥17; $P \leq 0.0473$) (Table 5.4). Nectar ingestion rate was either unaffected by type of visit or (on 3 of 6 sampling days) significantly higher for illegitimate flower visitors ($F \geq 15.7$; df=1,≥10; $P \leq 0.001$) (Table 5.4). Average time spent probing and ingesting per flower **(H)** was affected by type of bee visit (legitimate, illegitimate) ($F = 60.9$;

df=1,5; *P*=0.0006) but not day. On average, honey bees spent more time handling a flower during legitimate visits than during robbing (Table 5.3).

Foraging energy spent per flower was either unaffected by type of bee visit or (on 4 of 6 sampling days) higher by legitimate flower visitors ($F \geq 4.7$; df=1,≥10; $P \leq 0.0538$) (Table 5.4). There were significant effects of type of bee visit for net energy gain per flower on every sampling day ($F \geq 15.2$; df=1,≥10; $P \leq 0.0011$); however, the trend was inconsistent with higher net energy gain for legitimate visitors on four of six days and the reverse pattern the other two (Table 5.4). Energy spent per bee handling one flower was significantly affected by type of bee visit (*F*=60.9; df=1,5; *P*=0.0006) but not day; on average, honey bees spent more energy handling one flower during legitimate than illegitimate visits (Table 5.3). Net energy gain per sec handling time was either unaffected by type of bee visit or (on 3 of 6 sampling days) significantly higher for illegitimate over legitimate flower visitors ($F \geq 7.2$; df=1,≥17; $P \leq 0.0158$) (Table 5.4).

DISCUSSION

Nectar standing crop in intact and *X. virginica*-perforated flowers and quantity of nectar ingested by honey bees per *V. ashei* flower visit. Our results indicate that nectar standing crop per flower, whether measured by nectar volume (μl) or quantity (mg) of sugar, was higher in intact than perforated flowers, whether before or after one honey bee visit (Table 5.1). The most straightforward explanation is a higher frequency of bee visitation and nectar depletion in robbed (perforated) versus un-robbed (intact) flowers. Under ambient conditions honey bees strongly prefer perforated over intact flowers of *V. ashei* (Dedej and Delaplane 2004). Our findings with *V. ashei* are incongruent with the conclusion of Navarro (2001) that robbed flowers

in *Macleania bullata* Yeo do not differ in respect to nectar standing crop compared to un-robbed flowers, but are congruent with the results of Maloof (2001) who found less nectar in robbed (1.0 µl) versus un-robbed (4.8 µl) flowers of *Corydalis caseana* Gray and those of Stout et al. (2000) who found half the nectar in robbed (0.26 µl) versus un-robbed flowers (0.58 µl) of *Linaria vulgaris* P. Mill.

After one visit, honey bees removed a higher fraction of available nectar from perforated (% of volume, µl) than from intact flowers; the same held true for percentage of available sugar (mg) (Table 5.2). Other studies have reported similar divergences between robbed and un-robbed flowers. Stout et al. (2000) found that 8% of un-robbed flowers of *L. vulgaris* were empty compared to 56 % of robbed flowers. Maloof (2001) reported that in robbed flowers of *C. caseana* more than 20 % of the nectar remains after one bee visit. Such nectar residues help explain the high rates of successive visits we often note on individual florets. In general, we observed higher rates of successive visits with intact flowers compared to perforated ones, but we did not record these visits because of our *a-priori* assumption that honey bees collect all available nectar in flowers and avoid recently visited ones (Schmid-Hempel et al. 1985, Giufra and Nunez 1992, Nunez and Giufra 1996, Goulson et al. 1998, 2001). Stout et al. (1998) suggest that temporal repellence may depend on the density of foragers and degree to which they deplete floral resources.

Honey Bee Observations and Foraging Energetics. From the results presented in Tables 5.3 and 5.4 we summarize the following: Honey bees expressed a higher rate of *V. ashei* flower visitation if they engaged in nectar larceny (Table 5.4). These illegitimate visits were associated with comparatively reduced foraging time per flower, higher bee nectar ingestion rates (Table 5.4), and lower flower handling times (Table 5.3). When legitimate versus

illegitimate foragers are compared in terms of energetic parameters, there is either parity between the two or advantage for illegitimate visitors; the only parameter in which an apparent advantage was detected for legitimate foragers was net energy gain per flower which was higher for legitimate foragers on four of six sampling days (higher for illegitimate visitors the other two) (Table 5.4). For foraging energy spent per flower (Table 5.4) and handling energy spent per flower (Table 5.3) there was unambiguous advantage for illegitimate visitors, and for net energy gain per second handling time there was either parity or an advantage for illegitimate visitors (Table 5.4). Thus, we conclude that the majority of evidence indicates energetic advantage for honey bees that engage in nectar larceny in *V. ashei*.

The proximal causes of this advantage appear to be foraging and flower handling habits that result in comparatively higher rates of flower visitation and energy intake; in other words, short-tongued honey bees gain temporal energetic advantage by accessing the nectar in *V. ashei* corollae via the lateral perforations formerly made by *X. virginica*.

The distal cause is likely a flower morphology that impairs legitimate (aperture) access by *A. mellifera* to the basal nectaries of *V. ashei*. Lyrene (1994) suggested that *Vaccinium* spp. flowers with comparatively short and wide corollae and large apertures are more amenable to honey bee visitation. Flowers of *Vaccinium* spp. often express the unfavorable end of these spectra, especially depth of corollae at ca. 9-11 mm (Free 1993). European honey bees *A. mellifera* L., with tongue lengths of 5.7-6.6 mm (Winston 1987), are disadvantaged and sometimes compensate in other behaviors beside robbing. Honey bees have been reported to visit other Ericaceae (*Echium* spp.) with deep corollae by pushing their heads through the aperture to reach nectaries (Corbet et al. 1995). In our case, the lateral robbery holes made by *X. virginica*

afford easy access by *A. mellifera* to the basal nectaries of *V. ashei*, improving energetic profitability of a resource otherwise compromised by inconvenient morphology.

Nectar-collecting honey bees and bumble bees (*Bombus* spp.) are expected to forage in a manner that maximizes the rate of net energy intake (Hodges and Wolf 1981, Harder and Real 1987). In our case, this optimality was realized by the expression of secondary nectar larceny, which, in turn, actualized the dual benefits of increased flower visitation rates and (more variably) nectar ingestion rates. Flower visitation rate (Table 5.4) is likely a product of flower handling time per bee (Table 5.3); by probing lateral robbery holes, *A. mellifera* was able to reduce flower handling time by half. Concerning nectar ingestion rates (Table 5.4), on the six sampling days there was either parity between legitimate and illegitimate visitors, or higher ingestion rate for illegitimate. This was true in spite of the fact that the perforated flowers preferred by illegitimate *A. mellifera* visitors consistently held smaller rewards (Table 5.1). Honey bees realizing higher nectar ingestion rates are able to maximize their nectar loads; conversely bees with lower ingestion rates have extended foraging times and smaller lower crop loads (Nunez and Giurfa 1996). Farina and Wainselboim (2001) report that at higher feeder flow rates trained bees imbibe solution for a shorter period, but achieved a higher final load. Our results are consistent with these published observations.

The outcomes of the two dynamics of increased flower visitation rates and nectar ingestion rates translate to reduced foraging energy spent per flower (4 of 6 days) and, correspondingly, increased net energetic benefit per second handling time (3 of 6 days) for illegitimate foragers (Table 5.4). We note the incongruity posed by our results for net energy gain per flower in which legitimate foragers realized benefit 4 of 6 days. However, this anomaly

does not refute the general conclusion that illegitimate honey bee flower visitors enjoyed the greater energetic benefit in this study with rabbiteye blueberry, *V. ashei*.

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REFERENCES

Corbet, S.A., Saville N.M., Fussell M., Prys-Jones O.E and D.M. Unwin. 1995. The competition box: a graphical aid to forecasting pollinator performance. J. Appl. Ecol 32: 707-719.

Corbet, S.A. 2003. Nectar sugar content: estimating standing crop and secretion rate in the field. Apidologie, 34: 1-10.

Dafni, A. 1992. Pollination Ecology: a practical approach. Oxford University Press Inc., New York.

Delaplane, K.S., and D.F. Mayer. 2000. Crop pollination by bees. CABI Publishing, Oxon, UK, 344 pp.

Dedej, S., and K.S. Delaplane. 2004. Nectar-robbing carpenter bees reduce seed-setting capability of honey bees (Hymenoptera: Apidae) in Rabbiteye Blueberry *Vaccinium ashei* Reade 'Climax.' Environ. Entomol. 33(1): 100-106.

Faegri, K., and L.W. Van der Pijl. 1979. The principles of pollination ecology (third edition). Pergamon, Oxford, UK, 244 pp.

Farina, W.M., and A.J. Wainselboim 2001. Changes in thoracic temperature of honey bees while receiving nectar from foragers collecting at different reward rates. J. Exp. Biol. 204: 1653-1658.

Feurbacher, E., J. H. Fewell, P. S. Roberts, E. F. Smith, and J.F. Harrison. 2003. Effects of load type (pollen or nectar) and load mass on hovering metabolic rate and mechanical power output in the honey bees *Apis mellifera*. J. Exp. Biol. 296: 1855-1865.

Free, J.B. 1993. Insect pollination of crops. Academic, London

Gilbert, F.S., N. Haines, and K. Dickson. 1991. Empty flowers. Funct. Ecol. 5: 29-39.

Goulson, D., S. A. Hawson, and J.C. Stout. 1998. Foraging bumblebees avoid flowers already visited by conspecifics or by other bumblebee species. Anim. Behav. 55: 199-206.

Goulson, D., J.W. Chapman, and W.O.H. Hughes. 2001. Discrimination of unrewarding flowers by bees direct detection of rewards and use of repellent scent marks. J. Insect Behav. 14: 669-678.

Giufra, M and J.A. Nunez. 1992. Honey bees mark with scent and reject recently visited flowers. Oecologia 89: 113-117.

Harder, L. D., and L. A. Real. 1987. Why are bumblebees risk-averse? Ecol. 68:1104-1108.

Harder, L. D., and M.B. Cruzan. 1990. An evaluation of the physiological and evolutionary influences of inflorescence size and flower depth on nectar production, Funct. Ecol. 4: 559-572.

Harrison J.F., S. Camazine, J. H. Harden, S. D. Kirkton, A. Rozo, and X. Yang. 2001. Mite not make it home: tracheal mites reduce the safety margin for oxygen delivery of flying honeybees. J. Exp. Biol. 204: 805-814.

Heinrich, B. 1993. The hot-blooded insects. Strategies and mechanisms of thermoregulation. Harvard University Press, Cambridge Mass, USA.

Hodges, C.M., and L. L. Wolf. 1981. Optimal foraging in bumblebees – Why is nectar left behind in flowers? Behav. Ecol. Sociobiol. 9 (1): 41-44 1981.

Inouye, D. W. 1980. The terminology of floral larceny. Ecology 61:1251-1253.

Inouye, D. W. 1983. The ecology of nectar robbing. Pp. 153-173 *In* B. Bentley and T. Elias, editors. The biology of nectaries. Columbia University Press, New York, New York, USA.

Kearns, C. A., and Inouye D.W. 1993 Techniques for the pollination biologist. University Press of Colorado, Boulder, CO, USA.

Lyrene, P.M. 1994. Variation within and among blueberry taxa in flower size and shape. J. Am. Soc. Hortic. Sci. 119: 1039-1042.

Maloof, J.E. 2001. The effect of a bumble bee nectar robber on plant reproductive success and pollinator behavior. Am. J. Bot. 88: 1960-1965.

Maloof, J. E., and D. W. Inouye. 2000. Are nectar robbers cheaters or mutualists? Ecology 81:2651-2661.

Navarro, L. 2001. Reproductive biology and effect of nectar robbing on fruit production in *Macleania bullata* (Ericaceae). Plant Ecol. 152: 59-65.

Nunez, J.A., and M. Giufra. 1996. Motivation and regulation of honey bee foraging. Bee World, 77: 182-196.

Pleasants, J. M. 1983. Nectar production in *Ipomopsis aggregata* (Polemoniaceae). Am. J. Bot. 70: 1468-1475.

SAS Institute 1992. SAS/STAT user's guide, version 6. SAS Institute; Cary, NC, USA, 846 pp. (4^{th} edition).

Schmid-Hempel, P., A. Kacelnik, and A. I. Houston. 1985. Honeybees maximize efficiency by not filling their crop. Behav. Ecol. Sociobiol. 17: 61-66.

Schmidt-Nielsen, K. 1997. Energy Metabolism. *In*: Schmidt-Nielsen K (ed) Animal Physiology, Adaptation and environment, 5th ed, Cambridge Univ Press, UK pp 169-214.

Seeley, T. D. 1985. Honeybee ecology: a study of adaptation in social life. Princeton Univ Press, Princeton, NJ, USA.

Stout J.C., Allen J.A and D. Goulson. 1998. Repellent scent-marking of flowers by a guild of foraging bumblebees (*Bombus* spp.) Behav. Ecol. Sociobiol. 43 (4-5): 317-326.

Stout J.C., Allen J.A and D. Goulson. 2000. Nectar robbing, forager efficiency and seed set: Bumblebees foraging on the self incompatible plant *Linaria vulgaris* (Scrophulariaceae). Acta Oecologica. 21: 277-283.

Winston, M.L. 1987. The biology of the honey bee. Harvard Univ Press, Cambridge, MA, USA

Wolf, T.H.J., P. Schmid-Hempel, C. P. Ellington, and R.D. Stevenson. 1989. Physiological correlates of foraging efforts in honey-bees: oxygen consumption and nectar load. Funct. Ecol. 3: 417-424.

Table 5.1. Volume, sugar quantity, and sugar concentration of nectar standing crop in intact or perforated *V. ashei* flowers before or after being visited by one honey bee. Data were collected over six sampling days (Julian). Values are mean ± standard error, with *n* in parentheses. Differences between intact or perforated flowers within variable within day are indicated with different letters ($\alpha = 0.05$ level).

Julian day	Before one bee visit						After one bee visit					
	Volume of nectar (µl)		Quantity of sugar (mg)		Sugar concentration (%)		Volume of nectar (µl)		Quantity of sugar (mg)		Sugar concentration (%)	
	Intact	Perforated	Intact	Perforated	Intact	Perforated	Intact	Perforated	Intact	Perforated	Intact	Perforated
87	3.4 ± 0.5a (25)	1.9 ± 0.2b (25)	1.1 ± 0.2a (25)	0.5 ± 0.1b (25)	28.9 ± 1.3a (25)	25.5 ± 1.1b (25)	1.9 ± 0.3a (22)	0.3 ± 0.1b (20)	0.6 ± 0.1a (22)	0.07 ± 0.03b (20)	NA*	NA
88	4.7 ± 0.3a (20)	0.9 ± 0.4b (20)	1.5 ± 0.1a (19)	0.3 ± 0.1b (20)	26.2 ± 1.1a (19)	26.9 ± 0.9a (20)	2.7 ± 0.4a (20)	0.05 ± 0.02b (20)	0.9 ± 0.1a (20)	0.01 ± 0.01b (20)	30.6 ± 1.2 (20)	NA
91	2.8 ± 0.2a (23)	0.6 ± 0.1b (20)	1.4 ± 0.2a (22)	0.4 ± 0.1b (20)	50.1 ± 2.3b (22)	58.7 ± 0.2a (20)	1.3 ± 0.2a (10)	0.1 ± 0.03b (21)	0.8 ± 0.1a (10)	0.07 ± 0.02b (21)	NA	NA
92	2.6 ± 0.2a (25)	1.2 ± 0.1b (25)	1.0 ± 0.1a (23)	0.5 ± 0.05b (25)	32.8 ± 1.8b (23)	41.3 ± 1.3a (25)	1.7 ± 0.2a (25)	0.3 ± 0.06b (25)	0.7 ± 0.1a (25)	0.2 ± 0.03b (25)	33.9 ± 1.4b (25)	42.3 ± 0.2a (25)
94	4.9 ± 0.4a (25)	2.4 ± 0.2b (25)	2.5 ± 0.2a (25)	0.9 ± 0.1b (25)	40.3 ± 1.6a (25)	31.0 ± 1.5b (25)	2.6 ± 0.2a (25)	0.3 ± 0.07b (30)	1.4 ± 0.1a (25)	0.1 ± 0.03b (30)	43.4 ± 1.3a (25)	33.8 ± 1.0b (30)
104	1.8 ± 0.2a (41)	0.3 ± 0.9b (25)	0.7 ± 0.1a (41)	0.2 ± 0.03b (25)	33.6 ± 1.2b (41)	42.2 ± 0.9a (25)	1.7 ± 0.3a (22)	0.2 ± 0.04b (35)	0.6 ± 0.1a (22)	0.1 ± 0.01b (35)	30.6 ± 1.3a (22)	30.0 ± 0.4a (35)

*We do not report mean values for cells indicated 'NA' because flowers in those groups did not yield enough nectar to measure concentration. For subsequent energetic calculations requiring these values, however, we derived estimates based on average concentration values of the same type of flower for that day.

Table 5.2. Percentage of available nectar (µl), percentage of available sugar (mg), and absolute amount of sugar (mg) removed by one honey bee visit to *V. ashei*. Values are mean ± standard error, with *n* in parentheses. Means within a row followed by the same letter are not significantly different at the $\alpha = 0.05$ level.

	Type of flower	
	Intact	**Perforated**
Percentage of available nectar (µl) removed by one bee visit	37.9 ± 6.9b (6)	76.3 ± 9.0a (6)
Percentage of available sugar (mg) removed by one bee visit	36.1 ± 4.9b (6)	77.4 ± 7.4a (6)
Absolute amount of sugar (mg) removed by one bee visit	0.5 ± 0.1a (6)	0.4 ± 0.1a (6)

Table 5.3. Average time and energy spent by one honey bee during legitimate or illegitimate flower visits to *V. ashei*. Data were collected only for individual bees successively expressing one behavior type. Values are mean ± standard error, with *n* in parentheses. Means within a row followed by the same letter are not significantly different at the $\alpha = 0.05$ level. As no interactions were noted between type of bee visit and sampling day, data are pooled by type of visit.

	Type of bee visit on flowers	
	Legitimate	**Illegitimate**
Time (s) spent probing and ingesting per flower **(H)**	11.6 ± 0.6a (50)	5.8 ± 0.3b (52)
Energy (J) spent handling one flower ($\mathbf{k_h}$ H s)	0.8 ± 0.04a (50)	0.4 ± 0.02b (52)

Table 5.4. Average time and energy spent by one honey bee during legitimate (Leg) or illegitimate (Illeg) *V. ashei* flower visits for all days of measurements. Data were collected only for individual bees successively expressing one behavior type. As interactions between type of bee visit and sampling day were noted for these independent variables, data are presented by day. Values are mean ± standard error, with *n* in parentheses. Means within a row for each variable followed by the same letter are not significantly different at the $\alpha = 0.05$ level.

Julian day	Number flowers visited per min		Total foraging time (D + H) per flower (s)		Foraging energy (J) spent ($O_E = D_E + H_E$) per flower		Net energy gain (J) ($N_E = I_E - O_E$) per flower		Net energy gain (J) ($N_E = I_E - O_E$) per sec handling time		Nectar ingestion rate µl/s	
	Leg	Illeg	Leg	Illeg	Leg	Illeg	Leg	Illeg	Leg	Illeg	Leg	Illeg
87	2.6 ± 0.3b (5)	6.3 ± 0.9a (7)	25.6 ± 4.4a (5)	12.7 ± 3.9a (7)	1.8 ± 0.3a (5)	0.9 ± 0.3b (7)	9.2 ± 0.3a (5)	6.9 ± 0.3b (7)	0.8 ± 0.05a (5)	1.2 ± 0.1a (7)	0.1 ± 0.01b (5)	0.3 ± 0.02a (7)
88	2.5 ± 0.2b (10)	6.8 ± 0.6a (10)	26.2 ± 2.5a (10)	10.0 ± 1.5b (10)	1.8 ± 0.2a (10)	0.7 ± 0.1b (10)	7.7 ± 0.2a (10)	3.6 ± 0.1b (10)	0.8 ± 0.2a (10)	0.8 ± 0.1a (10)	0.2 ± 0.04a (10)	0.2 ± 0.02a (10)
91	3.3 ± 0.5a (5)	4.2 ± 0.4a (6)	20.6 ± 3.8a (5)	15.1 ± 1.8a (6)	1.4 ± 0.3a (5)	1.0 ± 0.1a (6)	8.8 ± 0.3a (5)	5.3 ± 0.1b (6)	1.1 ± 0.3a (5)	1.0 ± 0.2a (6)	0.1 ± 0.03a (5)	0.1 ± 0.02a (6)
92	4.0 ± 0.3a (10)	4.2 ± 0.5a (10)	16.1 ± 1.3a (10)	16.0 ± 1.7a (10)	1.1 ± 0.1a (10)	1.1 ± 0.1a (10)	4.4 ± 0.1b (10)	5.3 ± 0.1a (10)	0.5 ± 0.08b (10)	0.9 ± 0.1a (10)	0.1 ± 0.01a (10)	0.1 ± 0.02a (10)
94	2.6 ± 0.3b (10)	4.4 ± 0.7a (9)	26.3 ± 3.6a (10)	16.7 ± 2.6b (9)	1.8 ± 0.2a (10)	1.1 ± 0.2b (9)	17.2 ± 0.2a (10)	12.3 ± 0.2b (9)	1.4 ± 0.1b (10)	2.4 ± 0.4a (9)	0.2 ± 0.02b (10)	0.4 ± 0.05a (9)
104	4.2 ± 0.3b (10)	8.7 ± 0.5a (10)	14.9 ± 1.1a (10)	7.1 ± 0.4b (10)	1.0 ± 0.08a (10)	0.5 ± 0.03b (10)	0.7 ± 0.08b (10)	1.0 ± 0.03a (10)	0.1 ± 0.01b (10)	0.3 ± 0.02a (10)	0.01 ± 0.001b (10)	0.03 ± 0.002a(10)

CHAPTER 6

EFFECTIVENESS OF HONEY BEES IN DELIVERING THE BIOCONTROL AGENT *BACILLUS SUBTILIS* TO BLUEBERRY FLOWERS TO SUPPRESS MUMMY BERRY DISEASE[1]

[1] Dedej S., K. S. Delaplane, and H. Scherm. Submitted to *Biological Control*, 05/05/2004.

ABSTRACT

Honey bees are important pollinators of commercial blueberries in the southeastern United States, and blueberry producers often use supplemental bees to achieve adequate fruit set. However, honey bees also vector the plant pathogenic fungus *Monilinia vaccinii-corymbosi* which infects open blueberry flowers through the gynoecial pathway causing mummy berry disease. Here, we report the results of a 3-year field study to test the hypothesis that the use of bee hives equipped with dispensers containing the biocontrol product Serenade, a commercial formulation of the bacterium *Bacillus subtilis* which has shown activity against flower infection by *M. vaccinii-corymbosi* in previous laboratory experiments, can reduce mummy berry disease incidence when honey bees are used as pollinators in blueberries. Individual honey bees carried 5.1 to 6.4 $\times 10^5$ colony-forming units (CFU) of *B. subtilis* when exiting hive-mounted dispensers with Serenade. On caged rabbiteye blueberry bushes in the field, population densities of *B. subtilis* vectored by honey bees reached a carrying capacity of $<10^3$ CFU per flower stigma within 2 days of exposure, and there was a highly significant non-linear relationship between *B. subtilis* populations per stigma and bee activity, expressed as number of legitimate flower visits per time interval per cage ($R = 0.6928$, $P < 0.0001$, $n = 32$). Honey bee density (1600 or 6400 individuals per 5.8-m^3 cage) and Serenade treatment (presence or absence of the product in hive-mounted dispensers) significantly ($P < 0.05$) affected the incidence of fruit mummification on caged bushes, whereby increasing bee density increased disease incidence and application of Serenade reduced disease levels. For example, in the cages with 6400 honey bees, presence of Serenade reduced disease incidence from 21.1 to 6.6% in 2002 and from 66.5 to 43.5% in 2003. Taken together, results of this study suggest that use of a hive-dispersed biocontrol product such as Serenade as a supplement during pollination can reduce the risk of mummy berry disease.

This may be a prudent practice that optimizes the benefits to pollination of high bee densities while reducing the associated disease-vectoring risk.

KEY WORDS: Mummy berry, *Monilinia vaccinii-corymbosi*, biocontrol, *Bacillus subtilis*, honey bee, *Apis mellifera*, rabbiteye blueberry, *Vaccinium ashei*.

INTRODUCTION

The blueberry industry in Georgia and other southeastern states has expanded considerably during the past decade (Krewer and NeSmith 2002; Scherm and Krewer 2003). However, despite the steady increase in acreage, total blueberry production has remained variable among years. Failure to produce good blueberry yields is often the result of freeze injury during bloom (NeSmith 1999), poor pollination (Filmer and Marucci 1963, Lyrene 2004), and/or presence of disease (Scherm et al. 2001). Among the diseases affecting blueberry in Georgia, mummy berry, caused by an ascomycetes fungus named *Monilinia vaccinii-corymbosi* Reade (Honey), has the greatest economical impact on the industry (Scherm et al. 2001). Symptoms of the disease are manifested in the blighting of emerging leaves and shoots during early spring (primary infection) and in the mummification of maturing fruit in early summer (secondary infection). Fruit mummification results from the infection of open flowers by conidia of the pathogen via the gynoecial pathway (Batra 1983, Milholland 1977, Ngugi et al., 2002, Shinners and Olson 1996). These conidia are transported from blighted leaves, where they are produced, to the floral stigmas by wind, rain, and especially pollinating insects (Batra and Batra 1985, Cox and Scherm 2001, Woronin 1888). Among these pollinators, honey bees (*Apis mellifera* L.) have been reported to be the most numerous visitors on blooming blueberries in Georgia (Delaplane 1995). The involvement of pollinating insects in vectoring of *M. vaccinii-corymbosi* poses a dilemma for commercial blueberry producers: on the one hand, enhancing pollinator activity, most

commonly achieved by supplementing honey bees in the field (Scherm et al. 2001), is essential for ensuring effective pollination and adequate fruit set (Dedej and Delaplane 2003, Sampson and Cane 2000). On the other hand, increased bee density is likely to lead to increased vectoring of the mummy berry pathogen. Thus, strategies are needed to reduce the potential for disease transmission when honey bees are used as blueberry pollinators.

The disease is currently managed by a combination of pre-bloom and bloom sprays of fungicide (Scherm & Stanaland, 2001). While this control strategy is generally effective, repeated fungicide applications, which are necessary because of the protracted bloom of most rabbiteye blueberry cultivars, are expensive, time-consuming, and increase the likelihood of resistance development.

Despite the overall limited success of biocontrol in commercial horticulture, this pathosystem has several characteristics that suggest potential for success: (i) Fruit infection occurs during bloom, a defined period of host development. Hence, there is no need for season-long protection, which would be difficult to achieve with biological control. (ii) The stigma of the open flower, the only infection court for fruit infection, is covered with an exudate during the period of receptivity (Parrie & Lang, 1992). The moisture and nutrients provided by this exudate may provide a suitable environment for growth and multiplication of biocontrol agents. (iii) *M. vaccinii-corymbosi* germinates and infects slowly, suggesting that an effective biocontrol agent could preempt it. (iv) Bee pollinators target preferentially newly opened flowers.

We recently documented that the biofungicide Serenade, a commercial formulation of the bacterium *Bacillus subtilis* (Ehrenberg) Cohn, effectively controlled flower infection by *M. vaccinii-corymbosi* when applied directly to the stigmas of open flowers in the laboratory (Scherm et al. 2004). In these experiments, the biocontrol product was delivered manually in small quantities (~10 µg per stigma) as a wettable powder formulation using a transfer needle.

This type of dry-application could be mimicked in the field by using honey bees as vectors, given their documented ability to adsorb (Prier et al. 2001) and deliver formulated biocontrol agents in other pathosystems involving flower infection (Johnson and Stockwell 1998, Kovach et al. 2000, Maccagnani et al. 1999, Peng et al. 1992, Thomson et al. 1992, Yu and Sutton 1997). Bee delivery of Serenade to open blueberry flowers in the field could reduce flower infection by *M. vaccinii-corymbosi*, regardless of whether conidia of the pathogen are vectored by the bees themselves or by other means.

Based on these considerations, this study was undertaken to (i) determine the effectiveness of honey bees in transmitting *B. subtilis* from hive-mounted dispensers containing Serenade to open blueberry flowers in the field, and (ii) evaluate the efficacy of this biocontrol product vectored by honey bees in reducing the incidence of fruit mummification caused by *M. vaccinii-corymbosi*.

MATERIALS AND METHODS

Field site and experimental design. The study was carried out in a research blueberry planting at the Horticulture Farm of the University of Georgia (Oconee County) from 2001 to 2003. The planting was established in 1988 and consisted of alternating rows of 'Climax' and 'Premier' rabbiteye blueberry (*Vaccinium ashei* Reade). Maintenance of the planting, including fertilization, pruning, and weed control, followed commercially recommended practice (Austin, 1994). Plants remained untreated with fungicide and insecticide throughout the 3-year period. Supplemental overhead irrigation was applied as needed.

Experimental plots were delineated by caging blueberry bushes with 1.8 × 1.8 × 1.8-m frames covered with insect-proof Lumite screen (Bioquip, Gardena, CA). Each cage contained two adjacent 'Climax' plants along with two potted 2- or 3-year-old 'Tifblue' plants that served

as pollenizers. At the onset of bloom in early spring, bee hives containing target populations of 1600, or 6400 honey bees were placed into the cages. Bee populations were established by the gravimetric method of Delaplane and Hood (1997). Average population densities during the 3 years were 1178 ± 61.5 (mean ± SE, n = 10) and 4267 ± 85.4 for the 1600 and 6400-bee treatments, respectively, but for convenience we will refer to the treatments in terms of their initial target densities. Bee colonies were fed regularly with sugar syrup and were socially stabilized with synthetic queen mandibular pheromone (Bee-Boost; Phero Tech, Delta, BC, Canada) (Currie et al. 1994). The pheromone was used in lieu of a queen to eliminate confounding effects of differential brood production resulting from variable bee populations. Honey bee colonies were removed from the cages at the end of bloom.

Each bee hive was equipped with a hive-mounted dispenser (Gross et al. 1994) permitting bees to acquire the biocontrol product and to disseminate it within the cage. Dispensers were filled with Serenade (QRD 132 WP; Agraquest, Davis, CA) to a depth of about 0.5 cm, and the biocontrol product was replenished as needed. In 2001, the experiment consisted of two replicate cages for each bee density, all of which contained hive-mounted dispensers supplied with Serenade. In 2002 and 2003, six additional cages in which hives were not supplied with Serenade were included in the study. Thus, in the latter 2 years, the experiment included a second treatment factor, presence or absence of Serenade, in addition to the three bee density treatments described above.

Acquisition of *B. subtilis* by honey bees. In 2001, ten bees each from the cages with 1600 and 6400 honey bees were captured emerging from the hive dispensers. At the same time, an additional 20 bees were sampled at the apiary of the University of Georgia, located within 200 m of the experimental site, as they emerged from hives not supplemented with Serenade; these bees were used as a background control. Each bee was placed into a glass vial and killed by

freezing. Bees were washed individually in 5 ml of sterile potassium phosphate buffer (0.01 M, pH 6.7) for 15 s, the wash water was placed in a sonicating bath (Bransonic Model 2200, Branson, Shelton, CT) for 60 s to resuspend the bacteria, and population densities of *B. subtilis* were determined by dilution-plating in triplicate onto nutrient-yeast extract-dextrose agar (Lelliott and Stead 1987). Culture dishes were incubated at 23^{o}C, and the number of colony-forming units (CFU) of *B. subtilis* per bee was determined between 1.5 and 2 days later. Presence of *B. subtilis* was confirmed based on colony morphology.

Vectoring of *B. subtilis* to open flowers. In 2001 and 2002, individual flowers were sampled from within the cages and analyzed for population densities of *B. subtilis* on the stigmatic surface. In each cage containing Serenade, 40 to 50 unopened flowers were labeled on their corollas with a permanent marker and monitored for time of anthesis. Two days after opening and exposure to the bees within the cage, ten of these flowers were detached and assayed for population densities of *B. subtilis*. Styles were removed from the flowers and placed individually in 1 ml of sterile potassium phosphate buffer in microcentrifuge tubes, followed by vortexing for 15 s and incubation in an ultrasonic bath for 60 s. Serial dilutions were made and plated as described above. Population densities of *B. subtilis* were expressed as CFU per stigma. As bloom progressed, five and three independent experimental runs (consisting of non-overlapping 2-day exposure periods) were carried out in 2001 and 2002, respectively.

During each experimental run, honey bee activity (primarily a function of bee density and weather) was estimated by counting the number of legitimate flower visits on all bushes within each cage for one 2-min period per day during normal flight hours (11.00 to 16.00 h). Visits were considered legitimate if the bee probed the terminal aperture of the flower (Dedej and Delaplane 2003).

Incidence of fruit mummification. At the end of bloom in 2002 and 2003, the screens surrounding the cages were replaced with poultry netting to protect fruit from animals and unauthorized harvesting. When fruit were fully developed but still green, 30 fruit clusters were selected arbitrarily from each of the two 'Climax' bushes within each cage, and all fruit on these clusters were collected to determine the incidence of fruit mummification per bush. Each fruit was bisected individually, and the presence or absence of mycelium or pseudosclerotia of *M. vaccinii-corymbosi* was determined (Scherm and Copes 1999).

To obtain an estimate of disease pressure for the 2 years, incidence of fruit mummification was determined as described above for two "open plots" each year. Similar to the caged plots, the open plots consisted of two adjacent 'Climax' bushes; however, the bushes were not surrounded by screen cages, and no supplemental bees, Serenade, or potted pollenizer plants were added.

Statistical analyses. To quantify the Serenade-vectoring ability of honey bees, the relationship between population density of *B. subtilis* per stigma and bee activity was analyzed using non-linear regression analysis (SigmaPlot v. 8.02, SPSS, Chicago, IL) utilizing combined data from all eight experimental runs but omitting the data from control cages without bees. The regression model was of the form $y = a(1-b^x)$, where y is the population density of *B. subtilis* (CFU per stigma), x is bee activity (number of legitimate visits per 2 min per cage), and a and b are parameters to be estimated.

The effects of honey bee density, presence or absence of Serenade, and their interaction on the incidence of fruit mummification per bush were determined using two-way analysis of variance (ANOVA) for a completely randomized design (SAS v. 8.02, SAS Institute, Cary, NC).

RESULTS

Acquisition of *B. subtilis* by honey bees. Individual bees carried $5.1 \times 10^5 \pm 1.2 \times 10^5$ and $6.4 \times 10^5 \pm 5.3 \times 10^4$ CFU of *B. subtilis* (mean ± SE) when exiting hive-mounted dispensers containing Serenade in cages with 1600 and 6400 bees, respectively. No colonies of *B. subtilis* were obtained from control bees.

Vectoring of *B. subtilis* to open flowers. Bee activity varied between 0 and 48 legitimate visits per cage within a 2-min period, while population densities of *B. subtilis* reached within 2 days of exposure ranged from 0 to 5.1×10^3 CFU per stigma (Fig. 6.1). The regression between bee activity and bacterial population density was highly significant ($R = 0.6928$, $P < 0.0001$, $n = 32$) and indicated an apparent carrying capacity of $<10^3$ CFU per stigma attained for bee activities after around 10 visits per 2-min period per cage (Fig. 6.1).

Incidence of fruit mummification. The incidence of fruit infected by *M. vaccinii-corymbosi* was greater in 2003 than in 2002, presumably because of more abundant rainfall during spring of 2003. For example, average disease incidence in the most severely affected treatment (having 6400 bees per cage without Serenade application) was 21.1% in 2002 vs. 66.5% in 2003 (Fig. 6.2). Similarly, disease incidence in bushes in open plots (exposed to ambient bee activity without use of Serenade) was greater in 2003 (30.5%) than in 2002 (14.2%).

Both bee density and presence of Serenade significantly ($P < 0.05$) affected disease incidence in the 2 years (Table 6.1). In general, increasing bee density increased disease incidence, while application of Serenade reduced disease levels (Fig. 6.2). A significant bee density × Serenade interaction was observed however in 2002 (Table 6.1); this was due to absence of disease in the cages containing 1600 bees with no Serenade, while a low incidence of disease (3.4%) was observed in cages with the same bee density in the presence of Serenade

(Fig. 6.2A). In the cages having 6400 bees, application of Serenade reduced disease incidence from 21.1 to 6.6% in 2002 and from 66.5 to 43.5% in 2003.

DISCUSSION

This study shed light on several important aspects of the three-way interaction between a pollinator (honey bee), a flower-infecting fungus (*M. vaccinii-corymbosi*), and a bacterial biocontrol agent (*B. subtilis*) on blueberry in the field. First, we confirmed the role of honey bees in vectoring the pathogen, as evidenced by a higher incidence of fruit mummification in cages with higher bee densities. Secondly, we documented the ability of honey bees to acquire a commercial formulation of the biocontrol agent and vector it to open blueberry flowers where *M. vaccinii-corymbosi* infects. There was a positive, non-linear relationship between bee activity and the resulting population density of *B. subtilis* on the flower stigma. Thirdly, our results showed that bee-vectored Serenade significantly and consistently reduced the incidence of mummy berry disease. Taken together, results of this study suggest that application of Serenade via hive-mounted dispensers is a promising strategy to reduce the potential for transmission of mummy berry disease when honey bees are used as blueberry pollinators.

An active role of honey bees and other pollinating insects in vectoring conidia of *M. vaccinii-corymbosi* to open blueberry flowers has been documented previously. Indeed, pollinators are attracted to infected, conidia-bearing shoots by a sweet, almond-like odor (Batra and Batra, 1985; Woronin, 1888) and by a UV reflection pattern that mimics that of blueberry flowers (Batra and Batra, 1985). These earlier studies also documented the presence of conidia of *M. vaccinii-corymbosi* on the body parts of pollinating insects and a considerable reduction in the incidence of fruit mummification when pollinators were excluded from flowers (Batra and

Batra, 1985). The highly significant effect of bee density on disease incidence in our study is congruent with these reports.

In the present study, individual honey bees carried around 5×10^5 CFU of *B. subtilis* when exiting hive-mounted dispensers containing Serenade. This is similar to values reported for other formulated biocontrol agents such as *Trichoderma harzianum* (Kovach et al., 2000), *Gliocladium roseum* (Yu and Sutton, 1997), and *Pseudomonas fluorescens* or *Pantoea agglomerans* (Thomson et al., 1992) when vectored by honey bees and bumble bees. In a previous study with *B. subtilis* var. *niger*, honey bees acquired 2.1 to 3.2×10^3 spores of the bacterium in an aerosol wind tunnel experiment (Prier et al., 2001). The ability to adsorb and retain bacterial spores was hypothesized to be related to the bees' electrostatic charge (Prier et al., 2001).

As shown previously for other pathosystems (Johnson and Stockwell, 1998; Kovach et al., 2000; Maccagnani et al., 1999; Peng et al., 1992; Thomson et al., 1992; Yu and Sutton, 1997), this study documented the ability of honey bees to vector a commercially formulated biocontrol agent to open flowers of a target crop in the field. Indeed, population densities of *B. subtilis* on blueberry flower stigmas reached a carrying capacity of $<10^3$ CFU following bee transmission of Serenade. This is similar in magnitude to population sizes of 2.9 to 3.2×10^2 CFU for *P. fluorescens* on apple flowers (Thomson et al., 1992) and 0.7 to 1.7×10^3 CFU for *G. roseum* on raspberry flowers (Yu and Sutton, 1997) when honey bees were used to vector these biocontrol agents. Population densities of *B. subtilis* in our study were determined on flowers within 2 days of anthesis. This relatively short exposure period was selected because blueberry flowers are susceptible to infection by *M. vaccinii-corymbosi* only for a few days after they open (Ngugi et al., 2002); thus, reaching an adequate population density of the biocontrol agent within the first few days after anthesis is critical to provide effective control of the disease.

The ability of honey bees to vector *B. subtilis* was positively (but not linearly) related to honey bee activity. An average of 10 legitimate bee visits per cage in a 2-min period was required to reach the apparent carrying capacity of the biocontrol agent on flower stigmas. This level of bee activity is realistic for field conditions on uncaged blueberry bushes (Dedej, *unpublished*), although with progressing bloom period in rabbiteye blueberry, the proportion of legitimate visits decreases relative to that of illegitimate visits (Dedej and Delaplane, 2004). During illegitimate visits, honey bees rob nectar through lateral perforations in the corolla (created by carpenter bees, *Xylocopa virginica* L.), thereby avoiding contact with the stigma during visitation. Such change of behavior is likely to reduce the ability of honey bees to vector *B. subtilis* to the stigmatic surface in the field. Nectar-robbing behavior in the presence of carpenter bees is common in the southeastern United States (Cane and Payne 1990, Delaplane, 1995).

As expected (Batra and Batra 1985), higher honey bee densities increased the incidence of fruit infection by *M. vaccinii-corymbosi* in control cages without Serenade. When bee hives were supplied with the biocontrol product, however, disease incidence was reduced not only relative to levels measured in the control cages, but also to those in the open plots where bee numbers were not manipulated and where no Serenade was used. Thus, given the interest in increasing honey bee density in blueberry plantings to improve pollination (Dedej and Delaplane 2003), use of a hive-dispersed biocontrol product such as Serenade as a supplement during pollination can reduce the risk of mummy berry disease. This may be a prudent practice that optimizes the benefits to pollination of high bee densities while reducing the associated disease-vectoring risk.

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REFERENCES

Austin, M.E. 1994. Rabbiteye blueberries: Development, production, and marketing. Agscience, Auburndale, FL.

Batra, L.R. 1983. *Monilinia vaccinii-corymbosi* (*Sclerotiniaceae*): Its biology on blueberry and comparison with related species. Mycologia, 75: 131-152.

Batra, L.R., and S.W.T. Batra. 1985. Floral mimicry induced by mummy berry fungus exploits host's pollinators as vectors. Sci. 228: 1011-1013.

Cane, J.H., and J.A. Payne. 1990. Native bee pollinates rabbiteye blueberry. Alabama Agric. Exp. Sta. 37(4): 4.

Cox, K.D., and H. Scherm. 2001. Gradients of primary and secondary infection by *Monilinia vaccinii-corymbosi* from point sources of ascospores and conidia. Plant Dis. 85: 955-959.

Currie, R.W., M.L. Winston, and N.K. Slessor. 1994. The effect of honey bee (*Apis mellifera* L.) synthetic queen mandibular compound on queenless 'Disposable' pollination units. Am. Bee J. 134: 200-202.

Dedej, S., and K. S. Delaplane. 2003. Honey bee (Hymenoptera: Apidae) pollination of rabbiteye blueberry *Vaccinium ashei* var. 'Climax' is pollinator density-dependent. J. Econ. Entomol. 96: 1215-1220.

Dedej, S., and K. S. Delaplane. 2004. Nectar-robbing carpenter bees reduce seed-setting capability of honey bees (Hymenoptera: Apidae) in Rabbiteye Blueberry *Vaccinium ashei* Reade 'Climax.' J. Environ. Entomol. 33(1): 100-106.

Delaplane, K. S. 1995. Bee foragers and their pollen loads in South Georgia rabbiteye blueberry. Am. Bee J. 135: 825-826.

Delaplane, K.S., and W. M. Hood. 1997. Effects of delayed acaricide treatment in honey bee colonies parasitized by *Varroa jacobsoni* and a late-season treatment threshold for the south-eastern USA. J. Apic. Res. 36: 125-132.

Filmer, R.S., and P.E. Marucci. 1963. The importance of honeybees in blueberry pollination. 31st Ann. Blueberry Open House Proc., North Carolina Agric. Exp. Sta., pp. 14-21.

Gross, H.R., J. J. Hamm, and J. E. Carpenter. 1994. Design and application of a hive-mounted device that uses honey bees to disseminate *Heliothis* nuclear polyhedrosis virus. Environ. Entomol. 23: 492-501.

Johnson, K.B., and O. V. Stockwell, 1998. Management of fire blight: A case study in microbial ecology. Ann. Rev. Phytopathol. 34: 227-248.

Kovach, J., R. Petzoldt, and G. Harman. 2000. Use of honey bees and bumble bees to disseminate *Trichoderma harzianum* 1295-22 to strawberries for *Botrytis* control. Biol. Control 18: 235-242.

Krewer, G., and D. S. NeSmith. 2002. The Georgia blueberry industry: its history, present state, and potential for development in the next decade. Acta Hortic. 574: 101-106.

Lelliott, R.A., and D. E. Stead. 1987. Methods for the diagnosis of bacterial diseases of plants. Blackwell, Oxford.

Lyrene, P.M. 2004. Early-ripening rabbiteye blueberries. Pp. 113-118 in: Krewer, G. (ed.). Proc. Southeast. Blueberry Conf., 9-11 Jan. 2004, Savannah, GA. Department of Horticulture, University of Georgia, Tifton.

Milholland, R.D. 1977. Sclerotium germination and histopathology of *Monilinia vaccinii-corymbosi* on highbush blueberry. Phytopathology 67: 848-853.

Maccagnani, B., M. Mocioni, M. L. Gullino, and E. Ladurner. 1999. Application of *Trichoderma harzianum* by using *Apis mellifera* as a vector for the control of gray mould of strawberry: First results. IOBC Bull. 22:161-164.

NeSmith, D.S. 1999. Effect of bee pollination and GA_3 on fruit size and maturity of three rabbiteye blueberry cultivars with similar fruit densities. HortScience 34: 1106-1107.

Ngugi, H.K., H. Scherm, and J. S. Lehman. 2002. Relationships between blueberry flower age, pollination, and conidial infection by *Monilinia vaccinii-corymbosi*. Phytopathology 92: 1104-1109.

Parrie, E.J., G. A. Lang. 1992. Self- and cross-pollination affect stigmatic pollen saturation in blueberry. HortScience 10, 1105-1107.

Peng, G., J. C. Sutton, and P. G. Kevan. 1992. Effectiveness of honeybees for applying the biocontrol agent *Gliocladium roseum* to strawberry flowers to suppress *Botrytis cinerea*. Can. J. Plant Pathol. 14:117-129.

Prier K.R.S., B. Lighthart, and J. J. Bromenshenk. 2001. Adsorption model of aerosolized bacterial spores (Bacillus subtilis variety niger) onto free-flying honey bees (Hymenoptera: Apidae) and its validation. Environ. Entomol. 30 (6): 1188-1194.

Sampson, B. and J. Cane. 2000. Pollination efficiencies of three bee (Hymenoptera: Apoidea) species visiting rabbiteye blueberry. J. Econ. Entomol. 93: 1726-1731.

Scherm, H., and W. E. Copes. 1999. Evaluation of methods to detect fruit infected by *Monilinia vaccinii-corymbosi* in mechanically harvested rabbiteye blueberry. Plant Dis. 83: 799-805.

Scherm, H., R. D. Stanaland. 2001. Evaluation of fungicide timing strategies for control of mummy berry disease of rabbiteye blueberry in Georgia. Small Fruits Rev. 1(3), 69-81.

Scherm, H., and G. Krewer. 2003. Blueberry production in Georgia: Historical overview and recent trends. Small Fruits Rev. 2: 83-91.

Scherm, H., NeSmith, D. S., Horton, D. L., and Krewer, G. 2001. A survey of horticultural and pest management practices of the Georgia blueberry industry. Small Fruits Rev. 1(4): 17-28.

Scherm, H., H. K. Ngugi, A. T. Savelle, and J. R. Edwards. 2004. Biological control of infection of blueberry flowers caused by *Monilinia vaccinii-corymbosi*. Biol. Control 29: 199-206.

Shinners, T.C., and A. R.Olson. 1996. The gynoecial infection pathway of *Monilinia vaccinii-corymbosi* in lowbush blueberry (*Vaccinium angustifolium*). Can. J. Plant Sci. 76: 493-497.

Thomson, S., D. Hansen, and K. Flint. 1992. Dissemination of bacteria antagonistic to *Erwinia amylovora* by honeybees. Plant Dis. 76:1052-1056.

Woronin, M., 1888. Über die Sclerotienkrankheit der Vaccinieen-Beeren. Mem. Acad. Imp. Sci. St.-Pétersbourg (VIIe Série) 36: 1-49.

Yu, H., and J.C. Sutton. 1997. Effectiveness of bumblebees and honeybees for delivering inoculum of *Gliocladium roseum* to raspberry flowers to control *Botrytis cinerea*. Biol. Control 10:113-122.

Table 6.1. Analysis of variance to determine the effects of honey bee density, presence or absence of Serenade Biofungicide (*Bacillus subtilis* strain QRD132), and their interaction on the incidence of fruit mummification caused by *Monilinia vaccinii-corymbosi* on caged 'Climax' rabbiteye blueberry in the field.

		2002			2003		
Source	df	Mean Square	*F*	*P* > *F*	Mean Square	*F*	*P* > *F*
Bee density [a]	1	617.7	27.2	0.0002	5004.3	42.7	< 0.0001
Serenade [b]	1	137.9	6.07	0.0299	976.9	8.33	0.0137
Bee density × Serenade interaction	1	303.9	13.4	0.0033	216.8	1.85	0.1990
Error	12	22.7	---	---	117.3	---	---

[a] Bee densities were 1600 or 6400 individuals per 5.8-m^3 screen cage containing two mature test plants along with two potted pollenizer plants. Control cages without bees were not included in the analysis of variance.

[b] Applied via bee hive-mounted dispensers.

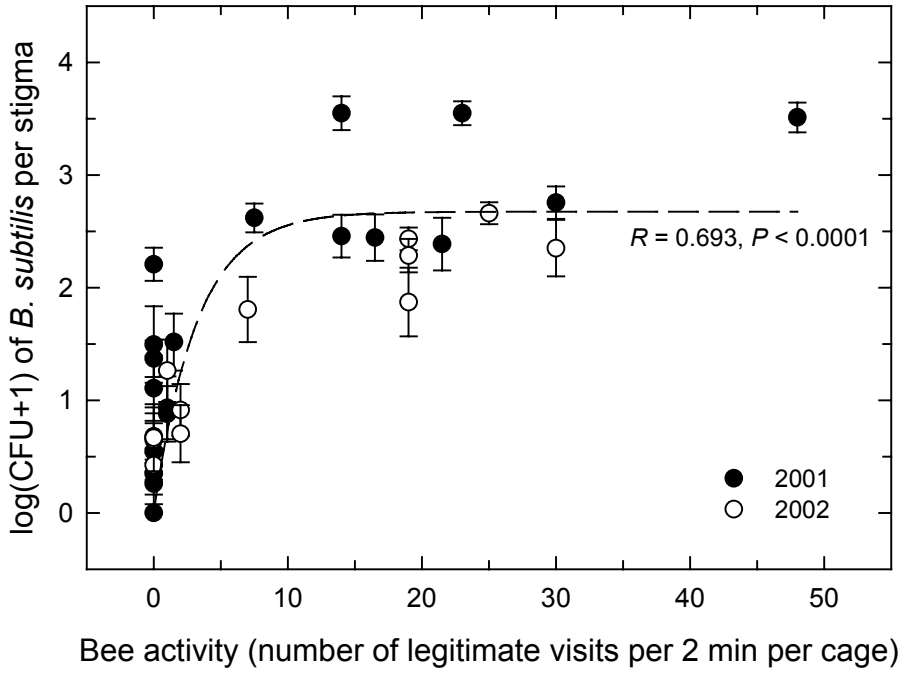

Fig. 6.1. Vectoring by honey bees of Serenade Biofungicide (*Bacillus subtilis* strain QRD132) from hive-mounted dispensers to the stigmas of open flowers on caged 'Climax' rabbiteye blueberry bushes in the field. Population densities of *B. subtilis* were determined by dilution-plating from individual stigmas 2 days after the flowers had opened; values are means and standard errors of ten flowers per cage. The regression equation is of the form $y = a(1 - b^x)$. There were five and three independent experiments in 2001 and 2002, respectively. CFU = colony-forming units.

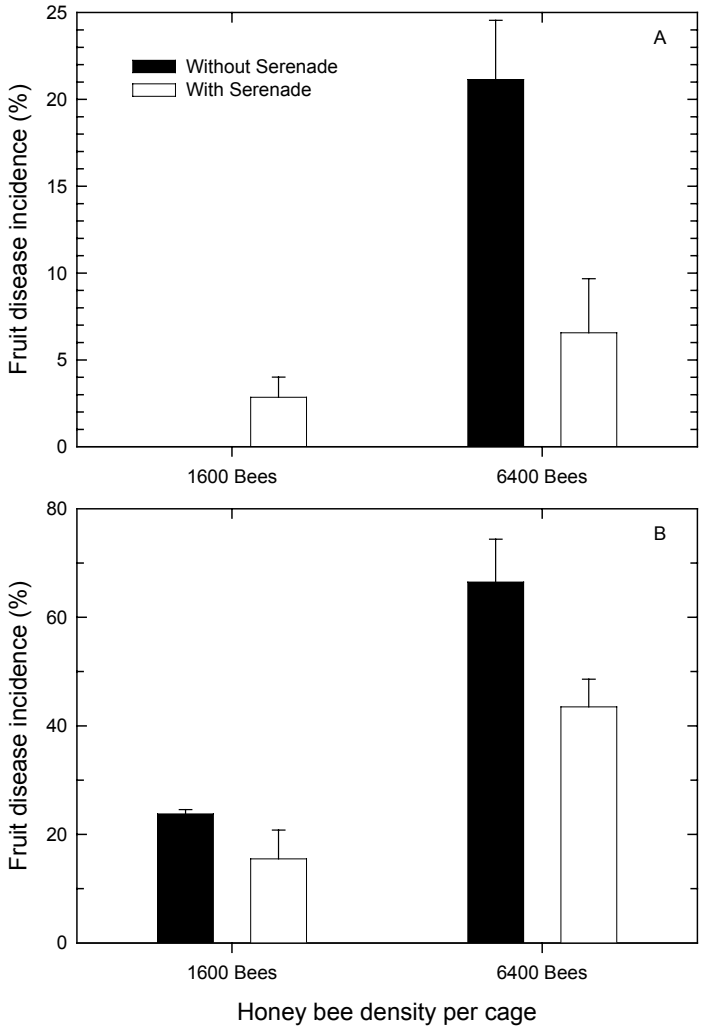

Fig. 6.2. Incidence of fruit mummification caused by *Monilinia vaccinii-corymbosi* on caged 'Climax' rabbiteye blueberry in the field in relation to honey bee density and presence or absence of Serenade Biofungicide (*Bacillus subtilis* strain QRD132) in hive-mounted dispensers within each cage. Values are means and standard errors of four bushes per treatment and are shown for experiments carried out in 2002 (**A**) and 2003 (**B**). Disease incidence in bushes without cages, supplemental bees, and no Serenade application was 14.2 ± 3.4% and 30.5 ± 2.8% in 2002 and 2003, respectively.

CHAPTER 7

CONCLUSION

Rabbiteye blueberry is an important energetic resource for pollinators. This study (Chapter 2) suggests that rabbiteye blueberry, a native crop species in the southeastern USA, is an important energetic resource for pollinators and its culture an important component of sustainable agriculture for this region.

Bush-type blueberries (*Vaccinium australe, V. corymbosum, V. ashei*; Ericaceae: Vaccinioideae) are an important commercial crop throughout much of the temperate world. *Vaccinium* spp. plants generally represent a good source of nectar and attract a diversity of nectarivores, especially bees (Hymenoptera: Apoidea). Despite the fact that many bee (Hymenoptera: Apoidea) species are attracted to rabbiteye blueberry flowers *Vaccinium ashei* Reade, there was formerly no descriptive information on the value of these plants as a floral resource for bees and other nectarivores. In this study (Chapter 2) conducted at a permanent blueberry orchard in Oconee County, Georgia USA, I collected data on the patterns of nectar production and sucrose concentration over flower life and blooming season, effects of weather on certain nectar characters, and potential nectar sucrose yield per ha of rabbiteye blueberry.

Nectar standing crop varied over the blooming season (Fig. 2.2) as well with age of flower (Table 2.2). Average nectar volume per flower was 2.85 µl, with an average sucrose concentration of 29.45%. On an individual flower basis, the average lifetime quantity of sucrose

produced was 1.89 mg, while the average quantity of nectar standing crop was 1.1 mg sucrose with a range of 0.05 – 4.56 mg. Nectar standing crop and nectar production during 24 h were affected by age of flowers and increased significantly in flowers older than one day (Tables 2.1 and 2.2).

I did not find effects of temperature, relative humidity, wind speed and solar radiation on quantity of sucrose produced by rabbiteye blueberry flowers, but I did find effects of these factors on nectar sucrose concentration (% Brix).

Air temperature for the 24 h preceding measurements had a complicated quadratic relationship with sucrose concentration (Fig. 2.3a), but air temperature for12 h preceding measurements varied negatively with sucrose concentration (Fig. 2.4a).

Sucrose concentration of nectar was inversely correlated with relative humidity 24 h (Fig. 2.3b) and 12 h (Fig. 2.4b) preceding measurements as well as at the time of measurements (Fig. 2.5a). Increase of solar radiation (Fig. 2.3c and Fig. 2.5c) and wind speed at the time of measurements (Fig. 2.5b) positively affected sucrose concentration of nectar.

Mature rabbiteye blueberry plants (1.5–1.8 m) yield a total seasonal nectar production of 130.75–156.91 L or 38.31–45.98 kg sucrose per ha. The total available nectar standing crop per ha at mid-anthesis is equal to 28.89–34.67 L which at 29.9 % sucrose represents 11.10–13.32 kg sucrose, enough to support 26–39 honey bee (*Apis mellifera* L.) colonies, 113–162 bumble bee (*Bombus* spp.) colonies, or 2020–2430 nests of *Osmia ribifloris*.

The effectiveness of *A. mellifera* as a pollinator of rabbiteye blueberry is bee density-dependent. Earlier work has shown that the effectiveness of *Apis mellifera* as a pollinator of rabbiteye blueberry is variety-dependent (Sampson and Cane, 2000). My data (chapter 3) indicate that *V. ashei* var. ‘Climax’ responds positively to increases in honey bee density as

measured by fruit-set, seed number, and speed of ripening. I concluded that honey bee pollination of *V. ashei* var. 'Climax' is also pollinator density-dependent.

Fruit set, seed number, and speed of ripening increased as bee density and flower visitation rates increased. More broadly, our results underscore the need to consider the pollinator densities achievable with candidate pollinator species. It is possible that a relatively inefficient pollinator, as determined by a lack of specialized behaviors or phenologies, may nevertheless be effective if it can field a forager force large enough to effect multiple flower visits.

The percentage of legitimate flower visits tended to increase as bee density increased within a range of 400 – 6400 bees; there were more legitimate visits in cages with 6400 bees than in those with ≤1600 bees (Table 3.1). Similarly, within a range of 400 – 6400 bees there was a trend for a corresponding increase in fruit set with means ranging from 25.0 – 79 percent (Table 3.1). The highest fruit-set was achieved in the open plots and in cages with ≥ 1,600 honey bees. When fruit-set was analyzed as a response variable related to log-transformed average bee density, the best fit was achieved with a quadratic model (Fig. 3.1). Regression analyses showed that fruit set increased linearly with the number of legitimate bee visits ($y = 34.7 + 1.6\,x$; $r^2 = 0.66$) (Fig. 3.2).

Mean weight of berries was unaffected by bee density but varied significantly between years. Within a range of 0 – 3200 bees the average seeds / berry tended to increase with increasing bee density; there were more seeds in berries in open plots than in cages with 12800 honey bees or ≤1600 bees (Table 3.1). These results are consistent with previous studies that found that blueberry fruits produce few seeds when insect pollinators are excluded (Lang and Danka 1991, Sampson and Cane 2000). When seed number was analyzed as a response variable

to log-transformed average bee density, the best fit was achieved with a quadratic model (Fig. 3.3). Declining seed numbers at upper bee density levels is best explained as an artifact of experimental conditions as described in chapter 3. When seed number was analyzed as a response variable to rate of legitimate bee visits (Fig. 3.4), the resulting best-fit model was linear, indicating no leveling off at upper levels of visitation, similar to the results for fruit-set. Indeed, one of the most notable features of this study is the general congruence of trends for fruit-set and seed number (compare, for example, Figs. 3.1 and 3.3 with Figs. 3.2 and 3.4).

Sucrose content ranged from 12.1 – 16.7 percent and fruits tended to be sweeter in cages with lower bee densities; percent sucrose was higher in cages with 400 bees than in those with ≥1600 bees or in open plots. Speed of ripening tended to be higher in cages with higher bee densities, however this trend was weaker than for the other variables; fruit ripening was faster in cages with 3200 bees than in those with ≤1600 bees or in open plots.

Nectar-robbing carpenter bees reduce seed-setting capability of honey bees in rabbiteye blueberry. Based on the results of a 2-yr study (chapter 4) conducted to assess how nectar robbing in honey bees affects fruit production in rabbiteye blueberry, and where various harvest parameters were measured from fruit collected from plants tented with: honey bees and carpenter bees (AX), carpenter bees (X), honey bees (A), no bees (0), or in open plots (open), I concluded that under tent conditions the continuous presence of 2 carpenter bees per 2 mature bushes results in nearly 40% incidence of *A. mellifera* robbery. This level of secondary nectar thievery does not reduce fruit-set but does reduce the number of seeds per berry – an important physiological indicator of pollinator efficacy.

These data confirm the high incidence of secondary nectar thievery by honey bees in southeastern blueberry plantations (Delaplane 1995) as well as the inability of honey bees to act

as primary nectar thieves. Nectar thievery by honey bees is dependent on the primary thieving activity of *Xylocopa* spp. during which the corollae receive the perforations that are subsequently preferred by honey bees.

Under ambient orchard conditions, temporal changes in the incidence of honey bee robbing behavior were explained by Mitscherlich asymptotic growth models (Fig. 4.1) in which rates of illegitimate honey bee flower visitation increase from initial lows to fixation at ≥95%. However, when the relationship between proportion of honey bee visits illegitimate and proportion of flowers perforated was examined independently of time, almost all the variation ($r^2 = 0.99$) was explained by a Mitscherlich asymptotic growth model (Fig. 4.2) which predicts that illegitimate foraging by honey bees increases from initial lows to fixation at ≥95% once the incidence of perforated flowers achieves 50% (x_p). Analyses suggest that the incidence of perforated flowers in an orchard rapidly achieves static levels, and honey bees learn quickly to preferentially visit flowers illegitimately, reaching near-fixation in the expression of this behavior.

Fruit-set in this study was affected by treatment (F=13.6; df=4,5; P=0.0068), but there were no year effects or interactions of treatment with year (F≤4.0; df=1 or 4,5; P≥0.0822). Fruit-set was significantly higher in the A, AX, and open plots than X or 0 plots (Table 4.1). These results support earlier work showing that *A. mellifera* is an effective pollinator of rabbiteye blueberry var. 'Climax' (Sampson and Cane 2000; Dedej and Delaplane 2003). The similarity in fruit-set between A and AX plots is noteworthy considering that the incidence of illegitimate flower visits by honey bees approached 40% in the AX plots (Table 4.1). Moreover, commercially acceptable yields are produced in orchards in which the incidence of illegitimate honey bee visitation meets or exceeds 95% (Fig. 4.1).

Even though fruit-set is similar in A and AX plots, seed numbers are significantly reduced in AX plots (Table 4.2). This, coupled with the fact that illegitimate flower visitation by honey bees was nearly 40% in the AX plots (Table 4.1), indicates that *X. virginica*-induced nectar robbery by *A. mellifera* compromises the pollination efficacy of *A. mellifera* in *V. ashei* as measured in seed-set.

Open-pollinated berries were larger than berries from all other treatments in 2001, while in 2002 berry weight followed the pattern A > open > AX > (X~0) (Table 4.2). Sucrose content of juice and speed of ripening were unaffected by treatments (Table 4.1).

Net energy advantage drives honey bees to nectar larceny in rabbiteye blueberry. In this study (chapter 5) was tested the hypothesis that honey bees after learning to visit perforations made in the lateral walls of the corolla by carpenter bees, switch behavior in response to an energetic advantage realized by illegitimate flower visits. Based on the results that on average, honey bees spent more time as well as energy handling one flower during legitimate visits than during robbing, I concluded that the majority evidence indicates an energetic advantage for honey bees that engage in secondary nectar thievery in *V. ashei*.

The results of this study indicate that nectar standing crop per flower, whether measured by nectar volume (µl) or quantity (mg) of sugar, was higher in intact than perforated flowers, whether before or after one honey bee visit (Table 5.1). Flower type (intact, perforated) affected the percentage of available nectar and sugar removed by one bee visit, but not absolute amount of sugar removed (Table 5.2). After one visit, honey bees removed a higher fraction of available nectar from perforated (% of volume, µl) than from intact flowers; the same held true for percentage of available sugar (mg) (Table 5.2). The most straightforward explanation is a higher frequency of bee visitation and nectar depletion in robbed (perforated) versus un-robbed (intact)

flowers. Under ambient conditions honey bees strongly prefer perforated over intact flowers of *V. ashei* (Dedej and Delaplane 2004).

Honey bees expressed a higher rate of *V. ashei* flower visitation if they engaged in nectar larceny (Table 5. 4). These illegitimate visits were associated with comparatively reduced foraging time per flower, higher bee nectar ingestion rates (Table 5.4), and lower flower handling times (Table 5.3). When legitimate versus illegitimate foragers are compared in terms of energetic parameters, there is either parity between the two or advantage for illegitimate visitors; the only parameter in which an apparent advantage was detected for legitimate foragers was net energy gain per flower which was higher for legitimate foragers on four of six sampling days (higher for illegitimate visitors the other two) (Table 5.4). For foraging energy spent per flower (Table 5.4) and handling energy spent per flower (Table 5.3) there was unambiguous advantage for illegitimate visitors, and for net energy gain per second handling time there was either parity or advantage for illegitimate visitors (Table 5.4).

The proximal causes of this advantage appear to be foraging and flower handling habits that result in comparatively higher rates of flower visitation and energy intake; in other words, short-tongued honey bees gain temporal energetic advantage by accessing the nectar in *V. ashei* corollae via the lateral perforations formerly made by *X. virginica*.

Nectar-collecting honey bees and bumble bees (*Bombus* spp.) are expected to forage in a manner that maximizes rate of net energy intake (Hodges and Wolf 1981, Harder and Real 1987). In my case, this optimality was realized by the expression of secondary nectar larceny which, in turn, actualized the dual benefits of increased flower visitation rates and (more variably) nectar ingestion rates. Flower visitation rate (Table 5.4) is likely a product of flower handling time per bee (Table 5.3); by probing lateral robbery holes, *A. mellifera* was able to

reduce flower handling time by half. Concerning nectar ingestion rates (Table 5.4), on the six sampling days there was either parity between legitimate and illegitimate visitors, or higher ingestion rate for illegitimate. This was true in spite of the fact that the perforated flowers preferred by illegitimate *A. mellifera* visitors consistently held smaller rewards (Table 5.1).

Honey bees realizing higher nectar ingestion rates are able to maximize their nectar loads; conversely bees with lower ingestion rates have extended foraging times and smaller lower crop loads (Nunez and Giurfa, 1996). Farina and Wainselboim (2001) report that at higher feeder flow rates, trained bees imbibe solution for a shorter period, but achieved a higher final load. Our results are consistent with these published observations.

The outcomes of the two dynamics of increased flower visitation rates and nectar ingestion rates translate to reduced foraging energy spent per flower (4 of 6 days) and, correspondingly, increased net energetic benefit per second handling time (3 of 6 days) for illegitimate foragers (Table 5.4).

Honey bees successfully vector the biocontrol agent *Bacillus subtilis* to blueberry flowers to suppress mummy berry disease. The results of this study (chapter 6) suggest that application of Serenade (QRD 132 WP; Agraquest, Davis, CA) via hive-mounted dispensers is a promising strategy to reduce the potential for transmission of mummy berry disease when honey bees are used as blueberry pollinators

Here are reported the results of a 3-year field study to test the hypothesis that the use of bee hives equipped with dispensers containing the biocontrol product Serenade, a commercial formulation of the bacterium *Bacillus subtilis* which has shown activity against flower infection by *M. vaccinii-corymbosi* in previous laboratory experiments, can reduce mummy berry disease incidence when honey bees are used as pollinators in blueberries.

This study shed light on several important aspects of the three-way interaction between a pollinator (honey bee), a flower-infecting fungus (*M. vaccinii-corymbosi*), and a bacterial biocontrol agent (*B. subtilis*) on blueberry in the field. First, was confirmed the role of honey bees in vectoring the pathogen, as evidenced by a higher incidence of fruit mummification in cages with higher bee densities. Secondly, was documented the ability of honey bees to acquire a commercial formulation of the biocontrol agent and vector it to open blueberry flowers where *M. vaccinii-corymbosi* infects. There was a positive, non-linear relationship between bee activity and the resulting population density of *B. subtilis* on the flower stigma (Fig. 6.1). Thirdly, the results of this study showed that bee-vectored Serenade significantly and consistently reduced the incidence of mummy berry disease.

Individual honey bees carried around 5×10^5 CFU of *B. subtilis* when exiting hive-mounted dispensers containing Serenade. This is similar to values reported for other formulated biocontrol agents (Kovach et al., 2000; Yu & Sutton, 1997; Thomson et al., 1992) when vectored by honey bees and bumble bees.

On caged rabbiteye blueberry bushes in the field, population densities of *B. subtilis* vectored by honey bees reached a carrying capacity of $<10^3$ CFU per flower stigma within 2 days of exposure (Fig. 6.1), and there was a highly significant non-linear relationship between *B. subtilis* populations per stigma and bee activity, expressed as number of legitimate flower visits per time interval per cage ($R = 0.6928$, $P < 0.0001$, $n = 32$).

Honey bee density (1600 or 6400 individuals per 5.8-m^3 cage) and Serenade treatment (presence or absence of the product in hive-mounted dispensers) significantly ($P < 0.05$) affected the incidence of fruit mummification on caged bushes, whereby increasing bee density increased disease incidence and application of Serenade reduced disease levels (Fig. 6.2). For example, in

the cages with 6400 honey bees, presence of Serenade reduced disease incidence from 21.1 to 6.6% in 2002 and from 66.5 to 43.5% in 2003.

REFERENCES

Dedej, S., and K.S. Delaplane. 2003. Honey bee (Hymenoptera: Apidae) pollination of rabbiteye blueberry *Vaccinium ashei* var. 'Climax' is pollinator density-dependent. J. Econ. Entomol. 96: 1215-1220.

Dedej, S., and K.S. Delaplane. 2004. Nectar-robbing carpenter bees reduce seed-setting capability of honey bees (Hymenoptera: Apidae) in rabbiteye blueberry, *Vaccinium ashei*, 'Climax.' Environ. Entomol. 33: 100–106.

Harder, L. D., and L. A. Real. 1987. Why are bumblebees risk-averse? Ecology 68:1104-1108.

Hodges, C.M., and L.L. Wolf. 1981. Optimal foraging in bumblebees – Why is nectar left behind in flowers? Behav. Ecol. Sociobiol. 9 (1): 41-44 1981.

Kovach, J., Petzoldt, R. and G. Harman. 2000. Use of honey bees and bumble bees to disseminate *Trichoderma harzianum* 1295-22 to strawberries for *Botrytis* control. Biol. Control 18: 235-242.

Lang, G.A., and G.R. Danka. 1991. Honey-bee-mediated cross-versus self-pollination of 'Sharpblue' blueberry increases fruit size and hastens ripening. J. Am. Soc. Hortic. Sci. 116: 770-773.

Sampson, B., and J. Cane. 2000. Pollination efficiencies of three bee (Hymenoptera: Apoidea) species visiting rabbiteye blueberry. J. Econ. Entomol. 93: 1726-1731.

Thomson, S., Hansen, D., and K. Flint. 1992. Dissemination of bacteria antagonistic to *Erwinia amylovora* by honeybees. Plant Dis. 76: 1052-1056.

Yu, H., and J.C. Sutton. 1997. Effectiveness of bumblebees and honeybees for delivering inoculum of *Gliocladium roseum* to raspberry flowers to control *Botrytis cinerea*. Biol. Control 10: 113-122.