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Characterization of early floral damage by cranberry tipworm (*Dasineura oxycoccana* Johnson) as a precursor to reduced fruit set in rabbiteye blueberry (*Vaccinium ashei* Reade)

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Abstract

The cranberry tipworm, *Dasineura oxycoccana*, is an important insect pest of rabbiteye blueberries, *Vaccinium ashei*, in the southeastern United States, with annual losses from infestation and damage exceeding $20 million USD. Using rabbiteye flowers from a range of developmental stages, we examined floral tissues microscopically to determine specific areas targeted by *D. oxycoccana*, as well as the impact of oviposition and larval feeding. Our results indicate that oviposition by *D. oxycoccana* occurs early in the growing season, as larvae were found exclusively between bracts of expanding inflorescences and the base of developing flowers. When bracts were removed, tissue necrosis resulting from larval feeding was found encompassing the calyx tube region near the pedicel. Such damage potentially leads to premature floral bud abscission, or aesthetically compromised fruit when mature. In the field, evidence of *D. oxycoccana* infestation would be undetectable until overlying bracts had abscised, and damage had already progressed to an extreme state.

Keywords: *Cranberry tipworm, blueberries,* Vaccinium, *development, floral injury*

1. Introduction

Vaccinium (Ericaceae) includes a number of economically important berry crop species throughout North America such as cranberries (*V. macrocarpon* Aiton), big huckleberries (*V. membranaceum* Dougl.), and lowbush (*V. angusti-folium* Aiton), highbush (*V. corymbosum* L.), and rabbiteye blueberries (*V. ashei* Reade). Although not affected by many of the common pests of small fruit, a number of insect species can cause considerable damage to plant foliage and floral structures (Palser 1961). Injury to plant structures, particularly flower parts, during the growing season can significantly affect the economics of fruit production as fruit may not develop from the damaged flowers, or fruit aesthetics may be adversely affected.

The cranberry tipworm, *Dasineura oxycoccana*, is the most important insect pest of rabbiteye blueberries, *Vaccinium ashei*, in the southeastern United States. The future of rabbiteye blueberry production is threatened by *D. oxycoccana* as Florida's commercial blueberry industry consists primarily of southern highbush and rabbiteye cultivars. For the past 20 years *D. oxycoccana* has reduced Florida's rabbiteye blueberry produc-tion by as much as 80%; total rabbiteye production in 2004 was less than 20%, compared with 85% in 1983. A review of southeastern US crop profiles for blueberries lists cranberry tipworm as a major pest that warrants attention, particularly with respect to detection and quantifying damages (NeSmith 1999). Economic losses directly associated with cranberry tipworm damage may exceed $20 million USD annually (OE Liburd pers. obs.).

Blueberries and cranberries are the only known hosts for *D. oxycoccana* (Gagne 1989), and until the mid-1990s tipworm damage to rabbiteye plantings had been mistaken for frost damage. While frost damage also causes floral and vegetative bud loss, Lyrene and Payne (1992) were the first to differ-entiate the effect of tipworm feeding on rabbiteye blueberry floral buds. *Dasineura oxycoccana* females oviposit between the developing floral and vegetative bud scales, and newly hatched larvae feed on the innermost meristematic tissues, reducing flower production and potential fruit yield by up to 80% (Lyrene and Payne 1992; Sampson et al. 2002). The loss of vegetative bud tissues can also negatively affect plant vigour, as well as susceptibility to secondary infection. Unfortunately, the management of *D. oxycoccana* using conventional strategies is

impracticable because larvae develop inside bud-scales, which protects them from conventional insecticide sprays.

In Florida, cranberry tipworm over-winter as larvae or pupae in the soil below the blueberry bushes, with adult flies emerging in late January to early February. The time of emergence is dependent upon temperature and moisture, but also cultural practices such as pruning and ground cover changes (Lyrene and Payne 1992; Lyrene 1995). Independent preliminary studies have alluded to high levels of *D. oxycoccana* early in the growing season (Liburd and Finn unpublished data). However, with limited literature concerning rabbiteye and *D. oxycoccana* plant–pest interactions, it is essential that the timing, injury, and biology be studied more thoroughly. Therefore, this study seeks to examine these factors by conducting histological and scanning electron microscopic examinations of oviposition and subsequent feeding events by *D. oxycoccana* on a variety of floral development stages in rabbiteye cultivars ('Climax' and 'Bonita'). The ultimate goal is to document injury so that effective management tactics can be developed to alleviate such damage to plant tissues.

2. Materials and methods

Inflorescences from rabbiteye cultivars 'Climax' and 'Bonita', at a variety of developmental stages (Spiers 1978; Figure 1), were randomly collected from a commercial blueberry farm in Windsor,

Florida. This field consists of nearly 10 acres of approximately 10-year-old rabbiteye varieties, with 'Climax' and 'Bonita' planted in alternating rows for cross-pollination. Each respective blueberry bush was approximately 1.5 m away from the next, while the two rows of distinct cultivars were separated by 3.5 m. Although they had been pruned occasionally over their 10-year history, the plants were approximately 2–3 m tall at the time of sampling, with each plant consisting of 20–100 canes arising from the crown.

Collections were made between 24 February 2003 (dormant and swelling buds) and 21 March 2003 (full bloom achieved). For each sampling date, specimens were collected from bushes with and without pollination bags (Delnet®, nonwoven poly-olefin plastic resin bags, Applied Extrusion Technologies Inc., Middleton, DE). Pollination bags were used as a control against insect infestation of plant tissues prior to collections. All specimens were collected in FAA fixative (45:2.5:2.5 ml of 50% ethanol:formalin:glacial acetic acid).

Specimens were transferred from FAA fixative and stored in 70% ethanol after arriving at Acadia University. Initially, a Nikon SMZ1000 dissecting microscope was used to determine levels of pest damage to the blueberry specimens, and to assist in dissections. Pictures of damage incurred by both rabbiteye cultivars were captured using a Nikon CoolPix995 digital camera mounted on the dissecting microscope. Images documenting the plant–pest interaction were obtained primarily from 'Climax',
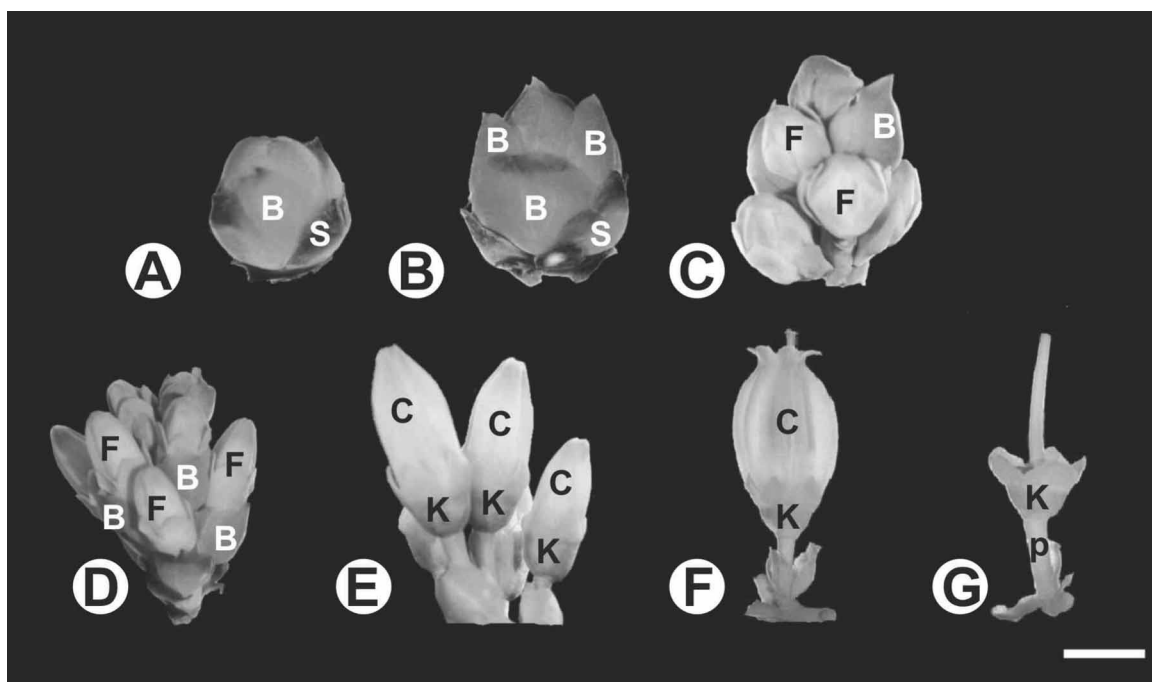

Figure 1. Blueberry inflorescence and floral development stages according to Spiers (1978). (A) Stage 1 – Inflorescence enclosed by bud scales (S). (B) Stage 2 – Inflorescence partially enclosed by bud scales, flowers covered by a large bract. (C) Stage 3 – Inflorescence with some bracts removed to show underlying developing flowers. (D) Stage 4 – Individual flowers expanded beyond bracts. (E) Stage 5 – Individual pre-anthetic flowers with elongate pedicels. (F) Stage 6 – Flowers at anthesis. (G) Stage 7 – Corolla dropped and beginning of fruit development. B = Bract; C = Corolla tube; F = Flower bud; K = Calyx tube; p = pedicel. Scale bars = 5 mm.

which was also the principal cultivar utilized for subsequent histological examinations.

2.1. Histological assessment of feeding

After initial observations were made concerning possible damage caused by insect pests, multiple representative samples of both damaged and undamaged specimens of all available stages of floral development were used for microscopic analyses. For both cultivars, serial sections from paraffin-embedded specimens were used to examine the extent of damage. All specimens were progressively dehydrated via a tertiary butyl alcohol series and then embedded in paraffin wax (Johansen 1940). Specimens were thin sectioned (10 μm) and affixed to microscope slides. Slides were subsequently deparaffinized with Histo-clear(and stained with Safranin-Fast Green following Johansen's method (Johansen 1940). Slides were viewed with transmitted light on a Zeiss Axioplan *2* microscope and images captured with a Diagnostic Instruments Spot II digital camera.

2.2. Scanning electron microscopic (SEM) investigations

In addition to histological investigations, SEM was utilized to provide higher resolution illustrations of damage incurred by the rabbiteye blueberries that could not be obtained using a dissecting microscope. Specimens were dehydrated through an ethanol series, critical point-dried in a Bio-Rad critical point-dryer, mounted on aluminum stubs, coated with gold palladium in a Hummer II (Technics) sputter coater, and viewed with a JEOL JSM-5900LV scanning electron microscope.

3. Results

A prolonged winter followed by warm temperatures resulted in a later break from dormancy and quicker bloom period for rabbiteye blueberry flowers in 2003. Collections obtained from both 'Climax' and 'Bonita' rabbiteye cultivars appeared to progress through floral development at similar rates, without discernable differences in susceptibility to insect pests or subsequent damage incurred after insect attack. Although the majority of specimens collected from both rabbiteye cultivars had not been targeted by *D. oxycoccana*, it was apparent that pollination bags did not deter insect infestation of rabbiteye blueberry flowers. Our collections revealed that *D. oxycoccana* larvae were present on the first sampling date, suggesting emergence and subsequent oviposition had commenced prior to first sampling.

Dasineura oxycoccana larvae were not observed in dormant (stage 1) rabbiteye blueberry inflorescences. Although only stage 2 and 3 inflorescences appeared to be targeted by *D. oxycoccana* during the collection period, evidence of feeding damage was visible in stages 2 through 4 (Figures 2 and 3). Initially, damage inflicted by tipworm was not apparent due to the tight packing of flowers and inflorescence bracts in stage 2 and stage 3 flowers (Figure 2A – C). When floral bracts were removed during dissection not only was feeding damage more visible, but *D. oxycoccana* larvae were still present depending upon the stage of floral development (Figures 2C and 3A,B). When found between inflorescence bracts and developing flowers *D. oxycoccana* larvae were consistently positioned perpendicular to the axis of the floral bud at the pedicel – receptacle junction (Figures 2A and 3A,B). Some inflorescences did not have larvae present, but possessed characteristic tipworm feeding damage (Figure 3C,D). Floral damage was only visible to the naked eye following abscission of bracts or sufficient elongation of floral bud pedicels (Figure 3C,D). Larvae appeared to feed only on the floral bud tissue in the area in which they were initially situated, but necrotic tissue resulting from their feeding appears to increase in size as damaged flowers develop (Figure 3C,D).

4. Discussion

Our study documents evidence of feeding injury in stages 2 and 3 of floral bud development for rabbiteye blueberries. It appears that stage 1 (Spiers 1978) inflorescence buds are too tightly packed for early emerging tipworm to oviposit between scales as neither eggs nor injury to floral tissues were observed. Typically, rabbiteye blueberry dormancy requirements are met in the south-eastern United States by early January, with 1 – 2 weeks of warm temperatures being sufficient to induce vegetative and floral bud swell (Spiers 1978). Because tipworm adults begin laying eggs within days of emergence (Lyrene 1995), high tipworm populations prior to bud swell are not necessary to have a considerable impact on rabbiteye floral buds. Finn (2003) and Lyrene and Payne (1995) found that few adults emerge prior to rabbiteye flowers passing through developmental stages 2 and 3. However, they were unable to identify any damage resulting from tipworm feeding. Although *D. oxycoccana* eggs were never observed in our collections, larvae of varying size were consistently found at the pedicel – calyx tube junction in stage 2 and 3 inflorescences (Figures 2A,B and 3A,B). The fact that feeding larvae were only found in stage 2 and 3 rabbiteye buds concurs with previous analyses (Lyrene and Payne 1995). Not only does this location offer protection to the feeding larvae, but also ample soft tissues for the larvae to feed on as they develop. With respect to the former, predators and contact insecticides, two of the most common form of pest management employed by Florida growers to combat tipworm (Finn 2003), are likely unable to target these pests in stage 2 and 3 inflorescences.

Obvious progression of *D. oxycoccana* injury was abundantly evident in collections of more mature inflorescences and floral buds. While early stages of
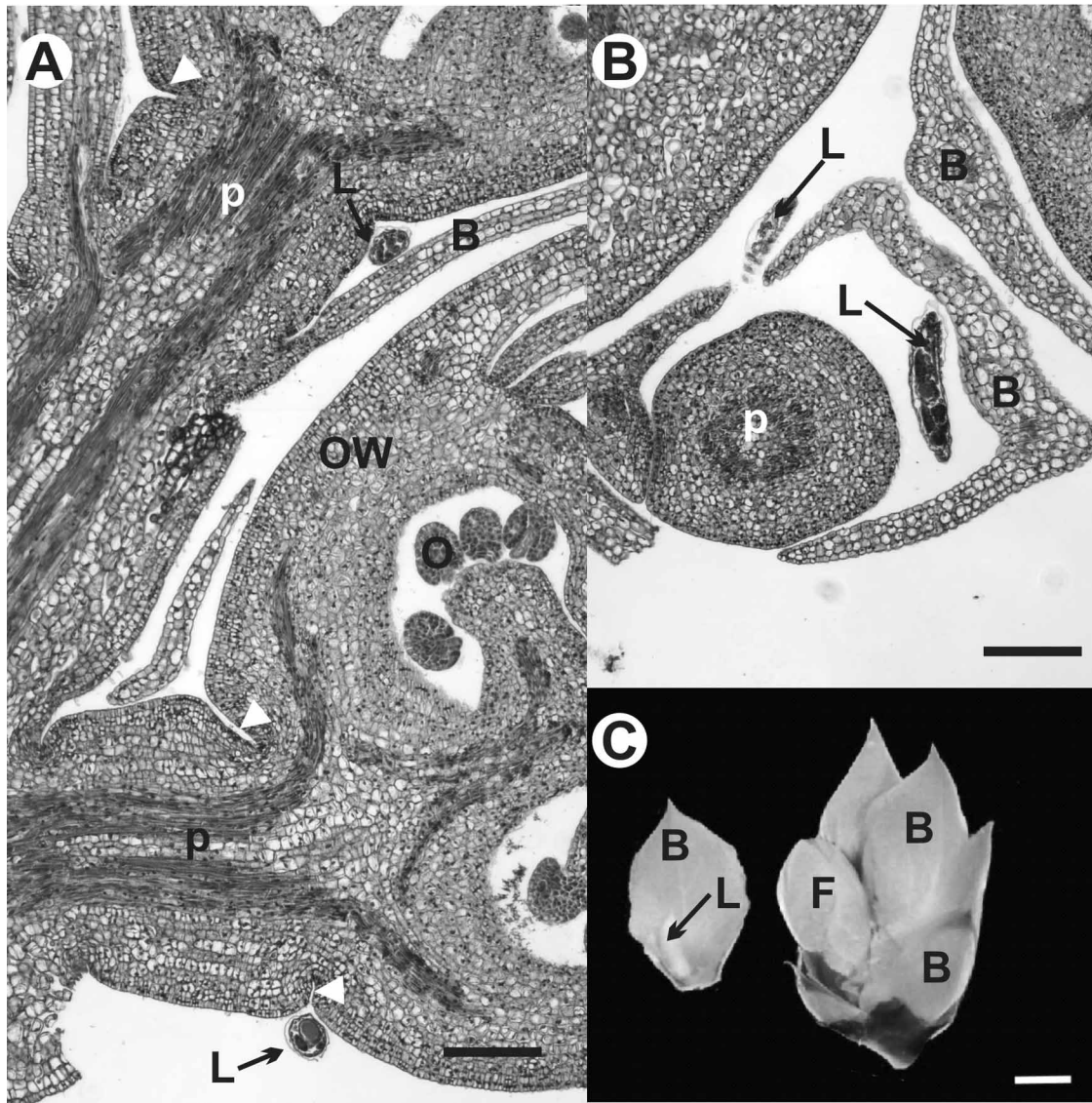
Figure 2. Paraffin-embedded and dissecting microscope images of larval blueberry gall midge within developing rabbiteye blueberry inflorescences. (A) Longitudinal section of a stage 2 'Climax' rabbiteye inflorescence illustrating *D. oxycoccana* larva position between bract and developing floral bud. (B) Transverse section of stage 2 'Bonita' rabbiteye floral bud pedicel illustrating gall midge larva placement between bract and developing floral bud. (C) Dissecting microscope image of stage 2 'Climax' inflorescence (right) displaying no obvious signs of larval *D. oxycoccana* infestation. However, bract removed from inflorescence (left) had *D. oxycoccana* larva within. B = Bract; F = Flower; L = Larva; OW = Ovary Wall; p = pedicel. Scale bars = 200 μm for A and B, 2 mm for C.

inflorescence development displayed minimal feeding injury (Figure 2C), brown, necrotic tissue was easily visible in later stages of floral development (Figure 3C,D). Individual flowers within infested inflorescences were consistently fed upon at the junction between the developing floral bud and the pedicel, with the resulting injury clearly affecting the attachment between the flower and pedicel (Figure 3C,D). This injury may be a contributing factor to extremely reduced crop loads in years when abundant tipworm populations and flower buds were at vulnerable stages coincide (Lyrene 1995).

Dasineura oxycoccana larvae were not observed in inflorescences later than stage 3. Flowers from inflorescence stages 5 through 7 did not demonstrate typical tipworm feeding damage (not shown), possibly because flowers may abscise before reaching these advanced developmental stages. Even if *D. oxycoccana* did not directly contribute to reduced crop production by causing premature floral bud abscission, the extent of the damage incurred in the calyx tube region in targeted floral buds would ultimately result in aesthetically compromised fruit.

Dasineura oxycoccana larvae are known to leave their original feeding location within rabbiteye inflorescences once they have completed their larval development and subsequently pupate in the soil where they can reside until the following growing season(s), or can emerge to give rise to subsequent generations within the current growing season (Gagne 1989). In the soil they are once again protected from airborne predators and aerially-sprayed contact insecticides until they emerge in subsequent seasons. Lyrene and Payne (1992, 1995)
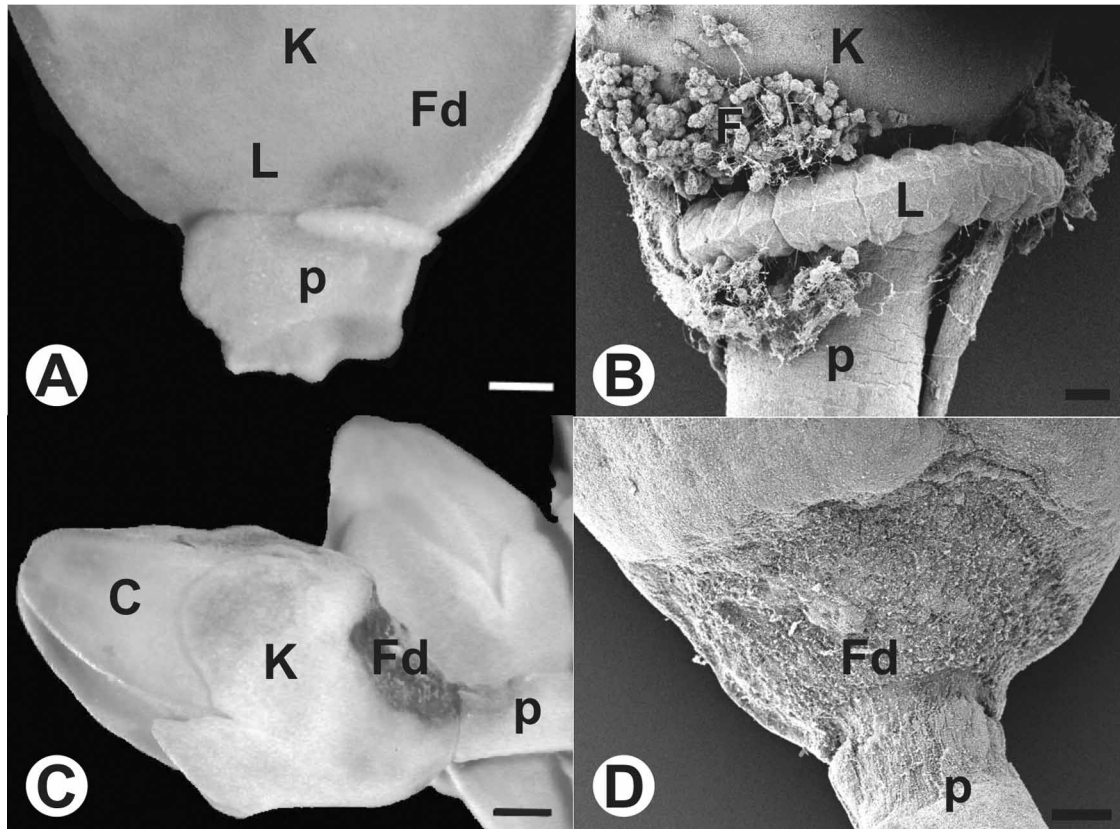
Figure 3. Dissecting microscope and SEM images illustrating position and extent of damage inflicted by *D. oxycoccana* in rabbiteye floral buds. (A) Early stage of *D. oxycoccana* feeding damage on a stage 2 'Climax' floral bud. (B) SEM of more mature *D. oxycoccana* larva on a stage 3 'Climax' floral bud surrounded by frass. (C) Characteristic *D. oxycoccana* feeding damage resulting in necrotic tissue on the pedicel and calyx tube of a stage 4 'Climax' flower. (D) SEM of necrotic tissue on stage 4 'Climax' floral bud caused by feeding damage on calyx tube and pedicel. B = Bract; C = Corolla; Fd = Feeding Damage; K = Calyx Tube; L = Larva; p = pedicel. Scale bars = 400 μm for A, 200 μm for B and D, 1.25 mm for C.

have reported that unmanaged infestations of cranberry tipworm can destroy up to 80% of floral buds in susceptible rabbiteye cultivars such as 'Climax' and 'Bonita', drastically diminishing potential fruit yields. In addition to floral bud damage, *D. oxycoccana* can damage vegetative meristems, with 'Climax' varieties of rabbiteye being one of the most susceptible to this particular damage. This damage greatly diminishes a plant's capability to produce sufficient spring foliage and growth which are ultimately required for proper development and support of fruit set later in the season. Symptoms of floral and vegetative damage include premature floral bud abortion, leaf curl, stunted growth, and blackened leaf tips (Liburd 2002).

Given that infestations are initially undetectable without dissection, and that larvae are protected from predators and pesticides due to the protective habitat of developing inflorescences, it is presumed that efforts towards managing tipworm in rabbiteye be directed towards targeting the pests prior to their emergence as adults. The extremely short 2–3-week development time for *D. oxycoccana* means that preventive measures aimed at emergence of the first generation of adults in a growing season should reduce damage in subsequent generations. The

conditions that foster adult emergence from pupation need to be determined to allow for the development of potential preventive measures prior to the date(s) or at the time when these conditions are met. Ultimately this cumulative knowledge should allow for more effective timing of insecticide applications if that is the mode of management chosen for use, and ideally fewer and smaller doses, by attacking the pest before it has the ability to infest many inflorescences.

4.1. Considerations for improving tipworm pest management

Insecticide pest management protocols used to combat tipworm infestation in rabbiteye cultivars implemented in 2002 and 2003 failed in the southeastern United States (Finn 2003). It may be that applications were simply not rigorous enough, or timing of application did not in fact correspond to the most susceptible developmental stages of rabbiteye blueberry inflorescence development. Of the various treatments employed, only diazinon and malathion (organophosphates) were effective in reducing *D. oxycoccana* (larval) infestation in stage 2 and 3 flowers (Finn 2003). However, US government regulations are beginning to restrict the use of

organophosphorous chemicals as broad-spectrum insecticides may have negative effects on beneficial pollinators, agricultural workers, and consumers.

An alternative strategy for consideration of *D. oxycoccana* control in Florida blueberry fields is the use of natural parasites such as eulophid wasps (*Aprostocetus*). Studies in Mississippi blueberry fields have demonstrated that eulophid wasps can decrease larval cranberry tipworm numbers by up to 75% between April and September (Sampson 2002). In northern Florida, tipworm appear to progress through multiple generations from January to early June, after which bud infestation ceases (Lyrene 1995). Thus, this or other comparable bio-control measures could help decrease the potential numbers of *D. oxycoccana* available to emerge the following season, thereby decreasing the potential for infestation.

Acknowledgements

References

Bosio G, Bogetti C, Brussino G, Gremo F, Scarpelli F. 1998. *Dasineura oxycoccana*, a new pest of highbush blueberry in Italy. Informatore Fitopatologico 11:36–41.

Finn E. 2003. Developing integrated pest management (IPM) techniques for managing key insect pests of blueberries in the southeastern United States. Gainesville: University of Florida. p 99.

Gagne RJ. 1989. The plant-feeding gall midges of North America. Ithaca, NY: Cornell University Press.

Johansen DA. 1940. Plant microtechnique. New York: McGraw-Hill.

Liburd O, Finn E. 2002. The status of blueberry gall midge in the southeastern United States. Gainesville, FL: University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS).

Lyrene PM, Payne JA. 1992. Blueberry gall midge: A pest on rabbiteye blueberry in Florida. Proceedings of the Florida State Horticultural Society 105:297–300.

Lyrene PM, Payne JA. 1995. Blueberry gall midge: A new pest of rabbiteye blueberries. Journal of Small Fruit & Viticulture 3(2/3):111–124.

NeSmith DSE. 1999. Blueberry research: 1999 annual research update. Gainesville: University of Georgia.

Palser BF. 1961. Studies of floral morphology in the Ericales. V. Organography and vascular anatomy in several United States species of the Vacciniaceae. Botanical Gazette 123(2):79–11.

Sampson BJ, Stringer SJ, Spiers JM. 2002. *Dasineura oxycoccana* (Diptera: Cecidomyiidae) in blueberry. Environmental Entomology 31(2):339–347.

Spiers JM. 1978. Effect of stage of bud development on cold injury in rabbiteye blueberry. Journal of the American Society for Horticultural Science 103(4):452–455.

Trinka DL. 1997. Production trends in the cultivated blueberry industry of North America. Acta Horticulturae (ISHS) 446:37–40.