Integrated Pest Management for *Dasineura oxycoccana* (Diptera: Cecidomyiidae) in Blueberry

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ABSTRACT The ecology and control for the little-understood, blueberry bud-infesting gall midge *Dasineura oxycoccana* (Johnson) was studied to help reduce an estimated 20–80% blueberry crop loss due to this insect in the southern United States. Principal natural enemies were eulophid wasps, 85% of which were *Aprostocetus* (Perkins). Overall parasitism rate was 7% in the field, at times peaking around 34%. A 75% decline in the abundance of larval *D. oxycoccana* coincided with parasitoid activity between April to September. Larval *Toxomerus geminatus* (Say) Metz (Syrphidae) were early-season predators of immature *D. oxycoccana.* Prey handling took 3–10 min, with each predator eating approximately seven gall midge larvae in a 16-h period. Prebloom applications of malathion would be effective larvicides against *D. oxycoccana*, inducing 94% mortality in 24 h. A microbial-based alternative to malathion, spinosad, induced average mortality of 46% in 24 h. Spinosad was as effective as phosmet (50% mortality in 24 h) for *D. oxycoccana* control. Patterns of host plant resistance to *D. oxycoccana* were not obvious among 26 cultivars, accessions and species of *Vaccinium.* Additionally, the use of a dormancy-breaking compound, hydrogen cyanamide, could also have a deleterious side-effect: boosting gall midge populations and spurring 50% greater infestation of *D. oxycoccana* larvae in rabbiteye blueberry buds.

KEY WORDS *Dasineura oxycoccana*, *Vaccinium*, Syrphidae, parasitoids, biological control, insecticides

THE OBJECTIVE OF this integrated pest management (IPM) study was to quantify the natural and anthropogenic factors contributing to the mortality of *Dasineura oxycoccana* Johnson (Diptera: Cecidomyiidae) in blueberry agroecosystems: natural enemy activity, insecticide toxicity, host plant resistance, and the impact of plant growth regulators. *Dasineura oxycoccana* has for the past 10 yr become a severe pest of cultivated blueberries grown along the U.S. Gulf Coast (Payne et al. 1988; Lyrene and Payne 1992, 1995). Important life history traits of *D. oxycoccana* have contributed to severe infestations and blueberry crop loss. Adult emergence is very early beginning around January or February in the southern United States, and as many as two to six generations are known to be produced per year on cultivated hosts (Roberts and Brodel 1990, Steck et al. 2000). Five to 10 eggs are laid between the separating scales of a swelling blueberry bud (Lyrene and Payne 1992). After eggs hatch, feeding on interior bud tissue by larvae induces necrosis and bud abortion (Gagné 1989). In Florida, heavy infestations have almost eliminated the production of rabbiteye blueberry, *Vaccinium ashei* Reade, as gall midges killed from 20 to 80% of the floral buds on susceptible bushes (Lyrene and Payne 1992, 1995). Damage to vegetative buds of rabbiteye and southern highbush blueberry cultivars distorts leaves and kills vegetative meristems, impairing a plant's ability to support a heavy berry crop (Driggers 1926, Gagné 1989, Lyrene and Payne 1992, Bosio et al. 1998, Steck et al. 2000).

Native natural enemies should rapidly build up in commercial fields where exposure to pesticides is minimal and *D. oxycoccana* hosts are reliably abundant. Larval predators of *D. oxycoccana* like *Toxomerus marginatus* (Say) (Syrphidae: Diptera) provide brief plant protection to cranberry (Mahr and Kachadoorian 1990, Voss 1996). Other natural enemies of *D. oxycoccana* in cranberry bogs include minute parasitoid Hymenoptera: a eulophid wasp, *Tetrastichus* sp. [=*Aprostocetus* (Perkins)], and two Proctotrupids: *Aphanogmus* Thomson and *Ceraphron pallidiventris* Ashmead (Barnes 1948). Although populations of tetrastichine eulophids are associated with *D. oxycoccana* infesting cranberries of the Great Lakes region and blueberries in Georgia (Barnes 1948; A. Amis and B. W. Wood, unpublished data), their field effectiveness, population dynamics and IPM value remain to be quantified.

Host plant resistance is a potentially useful pest management tactic for reducing the incidence of *D. oxycoccana* for cultivated blueberry. In Florida, where

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gall midge injury can be excessive, a blueberry-breeding program has incorporated the screening for host plant resistance to *D. oxycoccana.* Adequate global genetic resources exist for breeding *Vaccinium* cultivars resistant to *D. oxycoccana* attack. The USDA-ARS National Clonal Germplasm Repository presently conserves >1,300 accessions from 66 *Vaccinium* species worldwide. In the southern United States, blueberry species more susceptible to gall midge attack are rabbiteye blueberries, their hybrids with *V. constablaei* A. Gray, and northern highbush blueberries (*V. corymbosum* L.) (Lyrene and Payne 1995). Incorporation of alleles from diploid *V. darrowi* Camp, a wild, drought-tolerant evergreen shrub into northern highbush blueberry has produced an entirely new class of cultivated hybrid blueberries (southern highbush blueberry) having floral buds seemingly resistant to midge damage.

Growth regulators, in addition to affecting the target crop, can alter the expression of plant resistance by stimulating or inhibiting nontarget insect populations (Tingey and Singh 1980). Hydrogen cyanamide (H_2CN_2) is a plant growth regulator used to break bud dormancy and enhance blueberry yield in the southeastern United States (Saad 1992). It is conceivable that H_2CN_2 might also promote heavier bud-infestation by *D. oxycoccana* by stimulating buds to more rapidly reach stages more suitable to oviposition by female *D. oxycoccana.* H2CN2 might also induce an inhibitory response in *D. oxycoccana* because of its potent insecticidal properties (Chamberlain 1988).

Materials and Methods

Phenology and Natural Enemies of *D. oxycoccana.* Seasonal abundance of host larvae inside infested galls and emerging parasitoids were monitored for 499 d between March 1999 and September 2000 on a blueberry farm near Lucedale, Jackson County, MS. Terminal floral and vegetative bud galls were repeatedly sampled from five rows of 'Climax', five 'Premier' rows, two 'Tifblue' rows, two 'Powderblue' rows, and two 'Brightwell' rows. Initially, floral buds of *V. ashei* were the only oviposition sites available to female *D. oxycoccana.* Therefore, 40 damaged floral bud galls were collected from each blueberry row once flowering commenced. After bloom, female midges subsequently used leaf buds to lay eggs of which 15 galled leaf buds were taken per row. Larvae were removed from their galls by placing buds into polyethylene bags at 21°C. Larvae then naturally emigrated from their galls where they could easily be counted on the interior surface of the bag. All larvae inside each sample bag were counted on days 4 and 7. Voltinism for *D. oxycoccana* was calculated by dividing the interim period between emergence and winter diapause by the longest number of days it took gall midges to complete development inside the bags (≈28 d).

Emerging parasitoid wasps or parasitized larvae with wasp pupae clearly visible were counted about 20 d after bud collection. Other parasitoid specimens were reared from wild *V. elliottii* Chapm. galls from Pearl River County, and galls from cultivated southern highbush blueberry in Stone County, MS. The percent parasitism achieved by *D. oxycoccana* parasitoids was calculated as follows:

$$\% \text{ parasitism} = P/(M + P) \times 100,$$

where P is the total number of pupae or emerging parasitoid adults in a sample bag, and M is the number of unparasitized *D. oxycoccana* larvae (Kausalya et al. 1997). Synchrony in parasitoid-host emergence was determined by comparing respective rearing times for the parasitoid and host using one-way analysis of variance (ANOVA) (PROC GLM, SAS Institute 1985). Rearing time for parasitoids and their hosts was measured as the time from when buds were collected to when adult wasps and midges first appeared.

All larval *Toxomerus geminatus* (Say) Metz (Diptera: Syrphidae) that were encountered in the sample bags were removed and transferred to 5-cm-diameter petri dishes for feeding experiments at 21°C. A moist wad of tissue paper placed into each dish provided a refuge for each syrphid fly larva. We measured prey handling time, predation rates and predator longevity for *T. geminatus* (Begon and Mortimer 1986). Each predator was presented with a series of randomly selected, live *D. oxycoccana* larvae of known size. Multiplying body length (mm) and width at its widest points (mm) for *D. oxycoccana* larvae approximated prey size. The strength of the linear relationship between prey size and *T. geminatus* prey handling time was tested using PROC GLM (SAS Institute 1985).

Assessing the impact of prey density on the predation rate for *T. geminatus* larvae required randomly assigning predator larvae to feeding arenas (petri dishes) containing one of the three live *D. oxycoccana* prey levels (5, 10, or 15 prey larvae per dish). Predators were permitted to feed on mature larvae for 16 h per prey level. Each prey level was replicated six times and feeding occurred in darkness. The feeding rate or the type II functional response of *T. geminatus* to the varying prey densities was modeled using the Holling disk equation (PROC NLIN, SAS Institute 1985, Begon and Mortimer 1986, Fan and Petitt 1994):

$$Na = a \times N/(1 + a \times Th \times N),$$

where Na is the number of prey larvae killed, a is the attack rate (larvae per hour), N is the initial prey density, and Th is the estimated prey handling time (hours per prey larva). Life history data gathered for the field-collected larvae and laboratory-reared flies comprised observations on larval longevity (d) and the length of the pupal stage (d).

Christian Thompson (Diptera) and Michael Shauff (Hymenoptera) of the USDA-ARS Systematic Entomology Laboratory (SEL) identified representative specimens of *Toxomerus* and parasitoids. Some specimens sent to the USDA-ARS SEL in Beltsville, MD, were retained for the collection.

Insecticide Toxicity Trials and Chemical Control of *D. oxycoccana.* *D. oxycoccana* mortality induced by spinosad, malathion, and phosmet was tested using

bench-top toxicity bioassays. Commercial grade spinosad (SpinTor 2 SC, Dow Agrosciences, Indianapolis, IN) was mixed at a rate of 0.23 ml (AI)/liter of de-ionized water and was equivalent to a field rate of 375 ml product per hectare. Malathion 5 EC (Terra International, Sioux City, IA) was applied at a concentration of 5.1 g (AI)/liter of de-ionized water or at the recommended field rate of 3.4 kg 5 EC/ha. Phosmet (Imidan 70%WP, Gowan Company, Yuma, AZ) contained 2.8 g active ingredient per liter de-ionized water (field rate = 1.5 kg Imidan per hectare). 5% acetic acid was pipetted into the insecticide stock solutions to adjust the pH to 4.0 and prolong malathion and phosmet toxicity. All stock solutions were stored at 5°C and warmed to 21°C before starting the bioassays. Fresh stock solutions of pesticides were prepared as needed.

Dasineura oxycoccana larvae were collected in April 2001 from leaf bud galls on *V. ashei* bushes inside a screenhouse at the USDA-ARS Small Fruit Station, Poplarville, MS. The galls were placed into sealed plastic bags and water misted onto galls prevented gall midge larvae from desiccating. A replicated bioassay was arranged so as groups of approximately four to six gall midge larvae were each placed into 1 of 20 wells within a clear plastic microtiter plate. After larvae were transferred and healthy individuals counted, a pesticide treatment was randomly applied to a set of five wells. Sixty microliters of solution was placed into each well using an Eppendorf micropipette. The topical treatments were a de-ionized water control (11 replications), spinosad (10 replications), malathion (8 replications), and phosmet (8 replications). The percentage mortality after 24 h was calculated from the total number of larvae for five wells per treatment. Data on percentage mortality were corrected for background mortality using Abbott (1925) formula:

$$\text{Corrected \% mortality} = [(T - C)/(100 - C)] \times 100,$$

where T = % mortality due to the pesticide treatment, and C = % background mortality attributed to the de-ionized water control. Larval mortality for the three compounds was compared with the de-ionized water control using probit analysis (PROC PROBIT, SAS Institute 1985).

Host Plant Resistance. From the larval abundance samples collected from the Lucedale Farm, two indices were used to screen for host plant resistance of five different rabbiteye blueberry cultivars to bud infestation by larval *D. oxycoccana.* The first index, the average number of larvae to emerge per bud gall in each sample bag (larval density) was tested using a two-way ANOVA (PROC GLM) for the effects of bud type (i.e., floral or leaf bud), rabbiteye cultivar and their interaction on larval density (SAS Institute 1985). Dormant buds collected during the diapausal period for *D. oxycoccana* were excluded from this analysis. The other index, rearing time, or the average number of days after the buds were collected that adult gall midges first appeared was assessed by a one-way ANOVA (PROC GLM) that tested the relative impact of rabbiteye blueberry cultivar on *D. oxycoccana* emergence. Tukey's honestly significant difference (HSD) tests were used to separate means (SAS Institute 1985) for both ANOVA analyses.

Twenty-two different blueberry clones from replicated plantings near Wiggins (Stone County), as well as alternative reproductive hosts like wild *V. elliottii* near Poplarville (Pearl River County) and Lucedale (Jackson County, MS), and wild *V. stamineum* L. growing near Leakesville (Greene County, MS) were surveyed in 1999 to determine the host range for *D. oxycoccana.* The blueberry clones near Wiggins included one to four plants for a clone of wild *V. corymbosum* L., 12 clones and cultivars of southern highbush blueberry designated MS-47, MS-54, MS-60, MS-75, MS-78, MS-81, MS-91-4, MS-94-4, Magnolia, Pearl River, Cooper, and Gulf Coast, as well as the nine *V. ashei* cultivars 'Baldwin', Climax, Delite, Menditoo, Premier, Tifblue, Woodard, Centurion, and Bonita. Pedigrees for southern highbush blueberries were chiefly 25% *V. darrowi* and 75% *V. corymbosum*, except MS-47, MS-91-4, and MS-94-4 that had additional genes derived from *V. darrowi* and *V. atrococcum* (A. Gray) A. Heller. One-way ANOVA was used to test for differences among the different clones and cultivars. Tukey's HSD test detected any differences among mean densities of *D. oxycoccana* infesting buds where the level of significance was $P \leq 0.05$ (SAS Institute 1985).

Hydrogen Cyanamide. Hydrogen cyanamide (Dormex, SKW Trostberg AG, Trostberg, Germany) mixed with 0.5% Surf-Aid 80/20 a nonionic surfactant (Agro Distribution, LLC, Sioux City, IA) was applied to Climax rabbiteye blueberry bushes at a blueberry farm near Carnes (Forrest County, MS). Climax blueberry was chosen because it is commonly cultivated in the southern states, hydrogen cyanamide is used to enhance its leafing, and its floral buds are susceptible to *D. oxycoccana* infestation. Each of five pairs of Climax bushes received a specific H_2CN_2 treatment according to a randomized complete block design. The treatments were two unsprayed controls that were later pooled (N = 8 bush pairs), 1.0% (N = 4 pairs), 1.5% (N = 4 pairs), or 2.0% (N = 4 pairs) H_2CN_2 solution. Hydrogen cyanamide was applied to bushes to the point of runoff with pressurized backpack sprayers on 10 February 2000. Each of the five treatments was applied to four replicated rows and a two-bush buffer separated one treatment from another. Plastic sheets erected around bushes minimized drift during H_2CN_2 applications.

Flower buds killed by H_2CN_2 were scored as intact (0) or aborting (1). Multiplying the average score by 100 was equivalent to the % of floral buds that aborted on Climax bushes at various concentrations of H_2CN_2. Only intact Climax bud galls showing outward signs of gall midge feeding damage were collected. Larvae were extracted from intact *D. oxycoccana* galls collected from the treated and untreated plants. Galled buds were first collected to measure the initial larval infestation for Climax in buds on 10 February 2000 (1 h

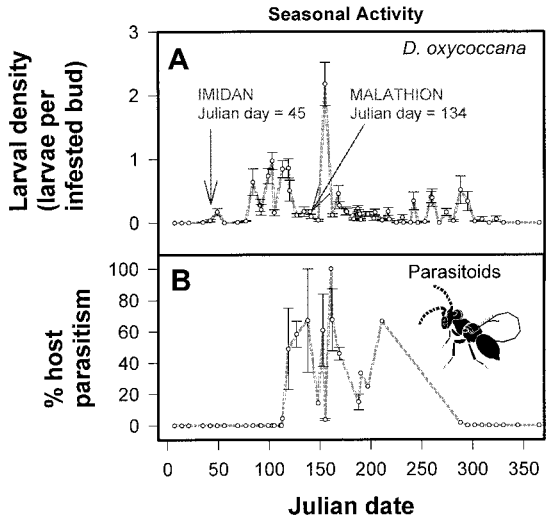

Fig. 1. Seasonal activity (years 1999–2000 combined) of (A) larval *D. oxycoccana* and (B) parasitoids of *D. oxycoccana* collected from five *V. ashei* cultivars planted near Lucedale, MS. Circles and bars represent mean ±1 SE. The arrows indicate when imidan and malathion insecticides were applied to rabbiteye blueberry bushes to specifically suppress gall midge populations.

before applying H_2CN_2). For each treatment within a replicate, 40 bud galls still attached to blueberry terminals were randomly picked from the two centermost bushes. Larval abundance was the total number of healthy, live gall midge larvae migrating from the 40 floral bud galls and into the interior of the sealed bag after 4 d of incubation at 21°C. New galls were collected 1, 2, and 3 wk after applying the H_2CN_2 treatments. Abundance was the average number of gall midge larvae per 40 bud galls collected for each treatment over the 3-wk period. Using the transformation $log_e (Y + 1)$ improved normality in the frequency distribution for the abundance of *D. oxycoccana* larvae. The effects of H_2CN_2 concentration, blocks (Climax rows), and their interaction on the relative abundance of *D. oxycoccana* were tested using two-way analysis of covariance (ANCOVA) with bud stage (Spiers 1978) as the covariate. One-way ANOVA differentiated between varying floral bud phytoxicity levels (% floral buds aborted) induced by elevated H_2CN_2 concentrations. Polynomial contrasts were used to identify linear, quadratic and cubic trends in the abundance of gall midge larvae and phytotoxicity at the different H_2CN_2 concentrations where $P \leq 0.05$ (PROC GLM, SAS Institute 1985).

Results

Phenology of *D. oxycoccana*. Adult gall midges emerged from winter diapause in early February (Fig. 1A) in SE Mississippi. Larval density per infested bud gradually increased during the pollination period from early February to early April as larvae fed on floral buds. Larval densities peaked in May and June after blooms faded and as leaf buds started to swell and unfurl. *D. oxycoccana* continued to feed on *V. ashei* for 300 d. Eleven or more generations were interpolated per year and a 2- to 4-wk periodicity in larval abundance clearly outlined the different *D. oxycoccana* generations (Fig. 1A). After two annual outbreaks, one in late winter/early spring and the other in summer/early fall, gall midge reproduction ceased during the winter from mid-November to late January (Fig. 1A). A prebloom application of phosmet (Imidan) intended to protect floral buds from *D. oxycoccana* on 14 February 1999 was effective at disrupting gall midge populations for about 4 wk on the Lucedale farm (Fig. 1A).

Natural Enemies of *D. oxycoccana*. After bloom and on 23 April (Julian day = 113) wasps began parasitizing larval *D. oxycoccana* infesting leaf buds. Percentage parasitism by wasps peaked on 9 July 1999 and totally subsided by 15 October 2000 (Fig. 1B). *Aprostocetus* (Perkins), *Quadrastichus* Girault (Eulophidae), platygasterid, and proctotrupoid wasps were parasitoids observed as pupae inside *D. oxycoccana* larvae or were reared as adults from bud galls. 85% of the wasps reared from larval *D. oxycoccana* were *Aprostocetus* sp. *Aprostocetus* developed singly within the bodies of their hosts. Before *Aprostocetus* wasps pupated, *D. oxycoccana* larvae had already left the galls and attained mature size. Parasitoid emergence was synchronized with their *D. oxycoccana* hosts: wasps emerged in 20 ± 12 d ($N = 48$) and unparasitized *D. oxycoccana* hosts emerged 20 ± 8 d ($N = 105$) after bud galls were collected ($F = 0.16$; df = 1, 152; $P = 0.6869$). An average of 34% (SE = 5) of *D. oxycoccana* larvae were parasitized during peaks in wasp activity. 7% (SE = 1) of all host larvae collected were parasitized during the entire 4-mo period that wasps were active. When parasitism occurred, field densities of *D. oxycoccana* decreased 75% and increased again by mid-September (Fig. 1A) as parasitoid activity subsided (Fig. 1B). On the 4 June 1999 (Julian day = 155), larval gall midge levels exceeded two larvae per bud, 3 wk after an unexpected application of malathion on 14 May 1999 (Julian day 134, Fig. 1A). This inflated resurgence in gall midge larval infestation was associated with a decline in the percentage of larval parasitism from 14 May (Julian day 134) to 4 June (Julian day = 155). Parasitism soon increased after 4 June with a corresponding decline in gall midge reproduction (Fig. 1B).

Toxomerus geminatus were predators that fed on gall midge larvae infesting terminals of *V. ashei*, *V. elliotii* and southern highbush blueberry from 1 April to 3 May 1999. *T. geminatus* larvae fully eviscerated gall midge larvae, sometimes consuming the entire exoskeleton. Observed prey handling times ranged from 3 or 4 min for small prey to 10 min for full-sized prey (Fig. 2). Expected prey handling (parameter *a*) that was estimated from the Holling disk equation took about 4 min ($F = 19.38$; df = 2, 18; $P < 0.0001$). Maximum predation rate for *T. geminatus* was approximately seven *D. oxycoccana* larvae eaten in 16 h and the time required to search for a single prey larva in

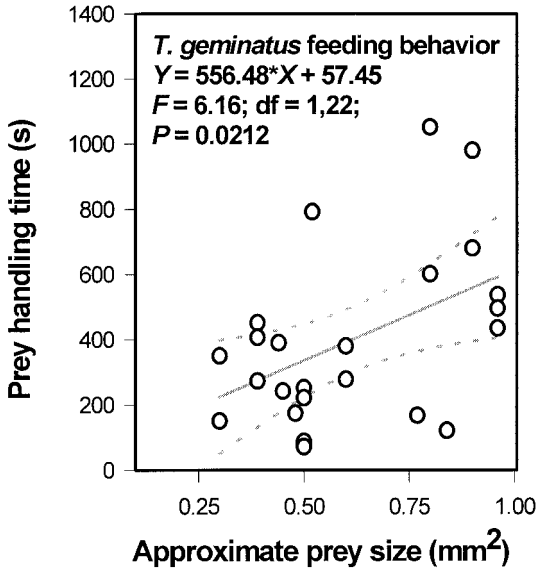

Fig. 2. Handling time (s) for laboratory-reared *T. geminatus* larvae preying on individual *D. oxycoccana* larvae of known approximate size (mm^2). Regression (solid) line, 95% CL (dashed lines), regression equation and *F*-test results are shown.

the dishes (parameter *Th*) was about 1 h (Fig. 3). Gall midge larvae, which are capable of catapulting themselves, made no attempts to escape predation from *T. geminatus* larvae. Up to 22 d of active feeding on larval *D. oxycoccana* was required before *T. geminatus* pupated (mean = 13.3 d, SD = 6.5, $N = 12$). 68% ($N = 13$ larvae) of the cultured *T. geminatus* larvae successfully pupated with puparia being attached to blueberry twigs and other substrates. Adults emerged 6–7 d later (mean = 6.5 d, SD = 0.5, $N = 12$).

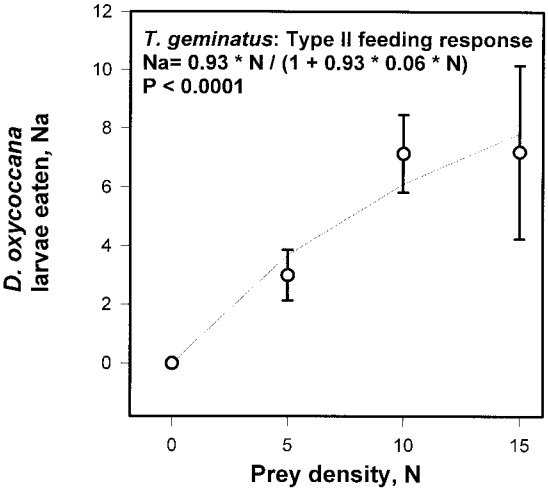

Fig. 3. Type II functional feeding response of unstarved *T. geminatus* larvae released inside feeding dishes along with three densities of *D. oxycoccana* prey. Circles represent mean prey consumed ±1 SE. The regression line for the Holling disk equation and the curve-fit probability value are given.

Insecticide Toxicity Trials. Accounting for natural mortality in the control using Abbott's formula, imidan induced 50 ± 6% mortality and spinosad killed 46 ± 6% of healthy gall midge larvae. These insecticides were more toxic than the water control and equally effective at inducing mortality in larval *D. oxycoccana* after 24 h of exposure (imidan: $\chi^2 = 34.86$, $P < 0.0001$, spinosad: $\chi^2 = 38.58$, $P < 0.0001$). Malathion was more toxic than the control and the other two insecticides evaluated in the bioassays inducing 94 ± 2% mortality after 24 h of exposure ($\chi^2 = 124.12$, $P < 0.0001$).

Host Plant Resistance. No clear pattern of host plant resistance to gall midge bud infestation could be identified in a wide variety of wild and cultivated southern *Vaccinium.* On average, bushes from five *V. ashei* cultivars hosted similar densities of *D. oxycoccana* larvae for 2 yr ($F = 1.35$; df = 4, 1,466; $P = 0.2513$). However, leaf buds harbored a 2-yr average of 0.27 larvae per infested bud ($N = 1{,}236$), almost twice that of floral buds, 0.15 larvae per bud, $N = 240$ ($F = 7.21$; df = 1, 1,466; $P = 0.0073$). Floral bud resistance to larval *D. oxycoccana* was not always linked to vegetative bud resistance for the five *V. ashei* cultivars. Leaf bud galls of Powderblue and Climax were more heavily infested than floral bud galls, whereas later-season 'Brightwell', Premier and Tifblue leaf and floral bud galls were equally infested throughout the year (Fig. 4A; $F = 3.26$; df = 4, 1,466; $P = 0.0112$). All five *V. ashei* cultivars were equally suitable developmental hosts for *D. oxycoccana*, with larvae taking from 18 to 23 d after the bud galls were collected to become adults (Fig. 4B; $F = 1.27$; df = 4, 94; $P = 0.2887$). Of the 259 adult *D. oxycoccana* captured alive from bud galls in the sample bags, 67% were females, 33% males.

In research plantings of blueberries in Stone and Pearl River Counties, gall midges were able to use as ovipositional hosts 22 different clones of wild *Vaccinium*, cultivated rabbiteye blueberry and southern highbush blueberry (Fig. 5). Gall midges were not detected within the flower or leaf buds of Baldwin (*V. ashei*) and MS-91-4 (a southern highbush blueberry). A wild population of northern highbush blueberry (*V. corymbosum*) was the most susceptible to larval gall midge infestation (Fig. 5; $F = 2.12$; df = 23, 358; $P = 0.0022$), but no differences were found among the other 21 clones infested by *D. oxycoccana* larvae at this site.

Hydrogen Cyanamide. A quadratic trend showed that the intermediate concentration of 1% (AI)/ H_2CN_2 boosted larval abundance 50% (Fig. 6; $F = 6.33$; df = 1, 63; $P = 0.0144$). At higher concentrations of H_2CN_2, larger percentages of floral buds aborted and disintegrated (Fig. 6; $F = 14.69$; df = 2, 232; $P < 0.0001$). Larval infestation of floral buds receiving 2% H_2CN_2 was similar to unsprayed buds. Gall midge infestation increased as bushes entered the later stages of bloom ($F = 41.57$; df = 1, 63; $P \leq 0.0001$) and larvae were more abundant in *V. ashei* buds collected from bushes in the two innermost rows ($F = 3.96$; df = 3, 63; $P = 0.0119$). There was no significant interaction be-

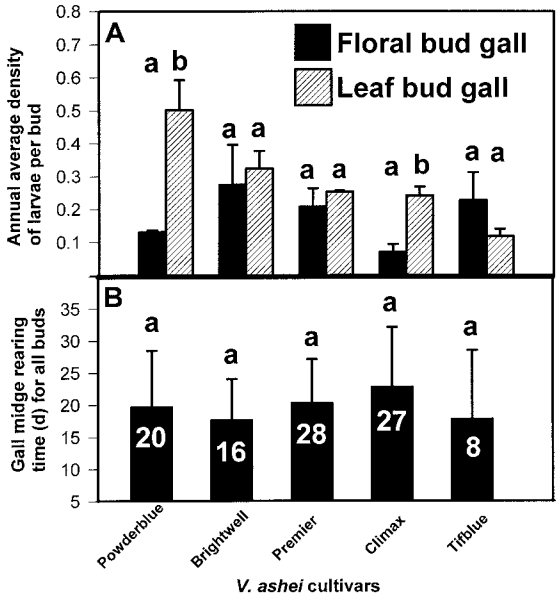

Fig. 4. Annual mean floral and leaf bud infestation by *D. oxycoccana* for the years 1999 and 2000 as well as the average rearing times for *D. oxycoccana* from bud galls gathered from five *V. ashei* cultivars planted on a farm near Lucedale, MS. (A) The average density of *D. oxycoccana* larvae infesting floral and leaf buds over a 2-yr period. Bars denote means ±1 SE. Letters that are different indicate differences in larval infestation between floral and leaf buds. (B) The rearing time for gall midges feeding from five *V. ashei* cultivars. Bars show mean ±1 SE and white numerals indicate the number of midges that we tracked throughout their development. There were no differences in gall midge development time among the five host cultivars using Tukey's HSD test ($P \leq 0.05$).

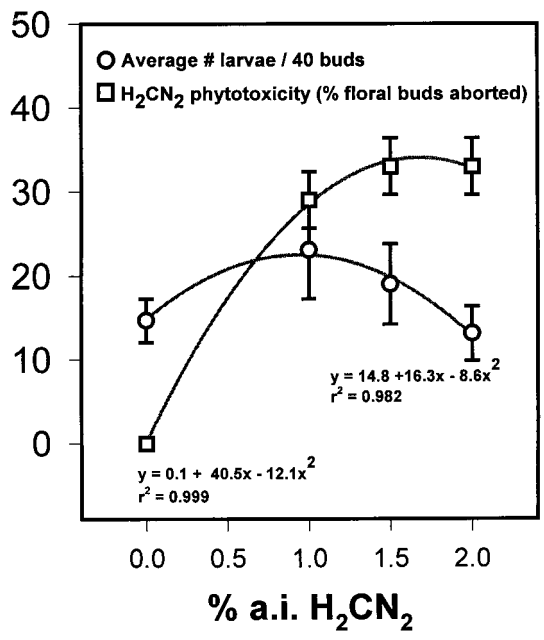

Fig. 6. Effect of varying hydrogen cyanamide concentrations (% [AI] H_2CN_2) on Climax floral bud infestation by *D. oxycoccana* (circles) larvae and floral bud phytotoxicity (squares) located at a blueberry farm near Carnes, MS. Symbols and bars are mean ±1 SE polynomial equations and r^2 values are given.

tween treatments and randomized blocks showing that larval gall midge responses were similar for the four H_2CN_2 concentrations, despite a greater abundance of larvae in buds clipped from bushes growing in the innermost Climax rows ($F = 1.02$; df = 9, 63; $P = 0.4322$).

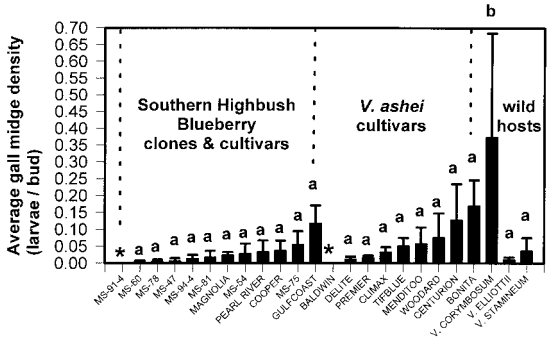

Fig. 5. Mean infestation by *D. oxycoccana* larvae at 24 clones of southern highbush, *V. ashei*, and three alternative wild hosts *V. corymbosum*, *V. elliottii* and *V. stamineum* from various counties in southeast Mississippi. Bars are mean ±1 SE. Letters that are different indicate mean differences using Tukey's HSD test ($P \leq 0.05$). Asterisks indicate plants of a particular clone were not infested with *D. oxycoccana* larvae.

Discussion

Improving Compatibility of Biological and Chemical Control for *D. oxycoccana*. Eulophid, platygasterid, and proctotrupoid wasps were parasitoids of the *D. oxycoccana* in Mississippi. These same groups parasitize a variety of cecidomyiid midges attacking sundry crops and wildplants (Barnes 1948, Wani et al. 1979, Gilstrap and Brooks 1991, Del Bene and Landi 1993, Doris et al. 1993, Lampo 1994, LaSalle and Delvare 1994, Kausalya et al. 1997, Shauff et al. 1997, Hinz 1998, Nwanze et al. 1998, Thompson et al. 1998). The eulophids *Aprostocetus* sp. and *Quadrastichus* sp. parasitized *D. oxycoccana* larvae inhabiting vegetative blueberry buds in Mississippi and have also been reared from blueberry floral buds in Georgia (A. Amis and B. W. Wood, unpublished data). The lower seasonal incidence in *D. oxycoccana* larvae from April to September appears to be more related to increasing and sustained parasitism than to an obligatory aestivation period for *D. oxycoccana* proposed by Steck et al. (2000). Endoparasitoid development was rapid for *Aprostocetus* and adult parasitoid emergence was synchronized with that of their *D. oxycoccana* hosts. Thus, a proposed schedule of targeting postbloom insecticide applications to match the 3- to 4-wk lifecycle of

D. oxycoccana (Lyrene and Payne 1995) could spur gall midge infestation by inadvertently removing as mortality factors parasitoids and other natural enemies. Eulophids reared from such minute hosts like *D. oxycoccana* larvae were not gregarious or prolific endoparasitoids, underscoring the need to safeguard fecundated parasitoids from pesticide overexposure as they actively seek hosts in spring and summer.

Adult *T. geminatus* are common floral visitors and their larvae serve as natural biological control in *Vaccinium* agroecosystems (Finnamore and Neary 1978, Duke 1980, Morse 1981, Kevan et al. 1983, Mohr and Kevan 1987, Horn 1988, Bugg et al. 1990, Voss 1996). *Toxomerus geminatus* larvae were sluggish predators, but voracious consumers of *D. oxycoccana* larvae (Van Driesche and Bellows 1996, Michaud 1998). Despite being exceptionally mobile, *D. oxycoccana* larvae made no effort to escape *T. geminatus* predators. In our feeding arenas, lengthier prey searching and handling limited to some degree the value of *T. geminatus* larvae as exclusive agents of biological control. In the field, however, higher concentrations of *D. oxycoccana* larvae as well as aphids on blueberry terminals would simplify prey searching by *T. geminatus.* Many mature gall midge larvae opting to drop to the ground to escape predation or pupate are likely to be killed by superabundant ground predators like red imported fire ants (*Solenopsis invicta* Buren) common to most blueberry fields along the U.S. Gulf Coast. *Toxomerus geminatus* larvae were also successfully reared on an exclusive diet of live *D. oxycoccana* prey, thereby increasing the chances of bolstering their field effectiveness through active management (Van Driesche and Bellows 1996, Gladis 1997).

Insecticide Control. Eulophid wasps and *Toxomerus* arrived too late or were too few to adequately protect the majority of the berry-producing floral buds from gall midge attack. A prebloom insecticide application would be required to protect the majority of earlier floral buds from burgeoning populations of *D. oxycoccana* in late winter and early spring. An increasingly larger array of insecticidal products is now applied against *D. oxycoccana*, as this pest's notoriety spreads throughout blueberry and cranberry growing regions of the United States. Phosmet, malathion, and more recently spinosad are among the three pesticides popularly applied for *D. oxycoccana* control. Malathion is an efficacious larvicide for *D. oxycoccana.* Malathion effectiveness increases when applied to smaller *D. oxycoccana* larvae (Mahr and Kachadoorian 1990). Phosmet was not the most toxic of the insecticides to larval *D. oxycoccana.* Residual phosmet activity briefly but obviously disrupted gall midge reproduction during the early bloom period for rabbiteye blueberries. Using phosmet or some other chemical as a prebloom adulticide and larvicide can delay the initial outbreak of *D. oxycoccana*, thereby lengthening the protective period for floral buds.

Additional toxicity bioassays are planned to help pinpoint effective rates of insecticides useful for managing insecticide resistance. The development of insecticide resistance is inherent in populations of *D. oxycoccana* because of the midge's shorter life cycle (3–4 wk), multiple generations, and continuous feeding for 10 mo of the year. In the early 1960s, resistance to DDT rapidly developed for New Jersey populations of *D. oxycoccana* infesting cranberries (Marucci and Moulter 1962). Spinosad is a naturalyte alternative to malathion and phosmet, it is as effective as phosmet, and is intended for managing insecticide resistance.

Host Plant Resistance. Developing resistant cultivars is often a more effective method than pesticides for gall midge control (Rechcigl and Rechcigl 2000) and has been successfully implemented against other insect species attacking blueberries (Ballington et al. 1993). *D. oxycoccana* infestations in cultivated fields likely first begin after midges emigrate from nearby overwintering hosts. Alternative hosts mainly include wild *Vaccinium* in Mississippi, but *Lysimachia* (Primulaceae) has been reported to be a nonericaceous host in northern cranberry bogs (Smith 1903). The mechanism responsible for resistance to *D. oxycoccana* has never been deduced in blueberries or cranberries. The degree of synchronization between blueberry floral bud expansion and adult midge emergence was ruled out as a reliable resistance factor (Lyrene and Payne 1995), but could be marginally important to earlier blooming varieties of blueberries. Floral buds of earlier flowering blueberry varieties to some extent escaped gall midge attack because midge populations had not yet reached full strength by the time floral buds were available for oviposition.

No evidence of antibiosis in buds of southern *Vaccinium* species was detected. A number of southern highbush, rabbiteye, and wild blueberry clones were equally infested with *D. oxycoccana* larvae, with the exception of an isolated clone of wild *V. corymbosum.* Blueberries adapted to the American South also have no apparent chemical defense against *D. oxycoccana.* Stable resistance to *D. oxycoccana* perhaps only occurs rarely in southern blueberry populations or spontaneously by selective breeding. Natural enemies, where sufficiently effective at reducing *D. oxycoccana* abundance, might also ease selection pressure for host plant resistance to develop (Pathak and Saxena 1980, Strong and Larsson 1992).

Hydrogen Cyanamide. Plant growth regulators applied to agricultural crops could affect host plant resistance because they are stimulated or are the byproducts of many gall-making insects (Byers et al. 1976, Wood and Payne 1988). The intended targets of the plant growth regulator, hydrogen cyanamide, are floral and leaf buds: the only ovipositional sites available to *D. oxycoccana.* In one experiment, use of hydrogen cyanamide to break blueberry bud dormancy and enhance leafing was shown to increase gall midge larval populations. Compared with unsprayed buds, hydrogen cyanamide at any of the application rates did not suppress natural larval populations of *D. oxycoccana.* Coupled with abortive bud loss due to increasing phytoxicity (Fallahi et al. 1998), greater bloom and berry loss is expected when hydrogen cyanamide is applied to blueberry bushes hosting locally large populations of *D. oxycoccana.*

In conclusion, IPM for southern blueberry must include the preservation of natural enemy activity and augmentation of their populations by the safe, precise, and effective timing of pesticide applications. Newer classes of biopesticides such as spinosad applied at higher rates are potentially effective products for chemical control and when rotated with other commonly used insecticides can help thwart insecticide resistance. Registered pesticides applied to southern blueberries just before first bloom will be effective at protecting a blueberry crop, although the length of this protection could vary with temperature and rainfall. Afterward, healthy populations of natural biological control organisms can be relied on to reduce gall midge infestations. IPM for *D. oxycoccana* can also be advanced by the ongoing search for resistant cultivars, and by understanding the impact of plant growth regulators on midge reproduction.

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