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INTRODUCTION

Lowbush blueberry is an economically important small fruit crop in Maine, New Hampshire, Massachusetts (to a lesser extent), and the Canadian Maritime Provinces. The value of lowbush blueberry in Maine in 1997 was ca. 65 million dollars. This is significant to the economy of Washington County, a county with a depressed economy where 60% of the blueberry production in Maine takes place (Yarborough 1997).

To maintain the economic viability of Maine's blueberry farms, to offer alternative pest control strategies in light of the implementation of the Food Quality Protection Act of 1996, and to reduce the human health and environmental risks associated with pest management, we have been researching biological control tactics. This technical bulletin outlines protocols and experimental design necessary for evaluation of entomopathogens targeted against the significant direct and indirect pests associated with lowbush blueberry.

Crop Origin and Production

Lowbush blueberry (also commonly referred to as wild blueberry) is one of four fruit crops native to North America (Yarborough 1997). It is an understory and forest gap complex of species adapted to temperate North America (Vander Kloet 1988), managed mostly for processed fruit and, to a lesser degree, fresh fruit (ca. 3% [Holbine 1994]). Six species in the genus *Vaccinium,* section *Cyanococcus* constitute what is referred to as lowbush blueberry (*Vaccinium angustifolium* Ait., *V. myrtilloides* Michx., *V. boreale* Hall and Alders, *V. darrowii* Camp, *V. tenellum* Ait., and *V. pallidum* Ait.) (Vorsa 1997), with *V. angustifolium* being by far the most abundant species in Maine and Maritime Canada (Smagula et al. 1997). Lowbush blueberry is a wild crop, not planted nor selected through breeding, but it is managed intensively throughout North America with respect to pruning, soil fertility, weeds, insect pests, and plant pathogens.

Native Americans were the first to encourage lowbush blueberry growth by periodically burning fields and gathering fruit the following year (Hawkes 1916; Kinsman 1986). Blueberries were a staple of many of the northeastern Native Americans because they could be dried and stored for long periods (Benson 1966). Early in U.S. history, settlers gathered berries as a public privilege on the blueberry barrens in northeastern Massachusetts and Maine (Yarborough 1997). In the United States, the lowbush blueberry industry grew rapidly during the civil war when blueberries were picked and shipped to union troops (Yarborough 1997). By 1930, in

Maine's Washington County alone, 40 canning factories were using the harvest from more than 100,000 ha (Phipps 1930).

Presently, more than 30 U.S. states and eastern Canada produce blueberries. The lowbush blueberry crop has tripled over the past 15 years and is now harvested on 52,800 ha in North America. Maine is the largest producer of lowbush blueberries in the world (43% of world crop: 25,000 ha yielding 28,500 metric tons), amounting to 20% of all blueberries in North America, cultivated (highbush, *V. corymbosum* and rabbiteye, *V. corymbosum* f. *ashei*) and wild (Yarborough 1997). Twenty percent of the combined wild and cultivated crop is produced in the Canadian provinces of New Brunswick, Newfoundland, Nova Scotia, Prince Edward Island, and Quebec. A minor percentage of lowbush blueberries is produced in New Hampshire, Massachusetts, Ontario, and Saskatchewan.

Crop Characteristics

Lowbush blueberries are perennial shrubs (up to six species of *Vaccinium*) that spread by underground rhizomes, with aerial shoots occurring every 2 to 30 cm. Genetic outcrossing and sexual reproduction does occur between mostly diploid clones (although polyploid hybrids exist [Vander Kloet 1988]) and can result in plants becoming established from seed. However, vegetative growth via rhizomes is the predominant mechanism of spread. Because of this, fields are comprised of many patches of genetically identical clones (Smagula et al. 1997), resulting in considerable within and between field heterogeneity in stem density, stem height, leaf trichome density, leaf color, fruit buds per stem, blossoms per fruit bud, fruit size, and fruit quality. Lowbush blueberry flowers are protandrous (the pollen is ready for dispersal a few days before the stigma becomes receptive) and thus are dependent upon insect pollination, mostly by bees.

There are 40 species of bees associated with lowbush blueberry in Maine (Stubbs et al. 1993; Drummond and Stubbs 1997). In fact, pollination is considered the most important input in blueberry production (Eaton and Murray 1997), and renting honey bee hives is the largest single production cost (Stubbs and Drummond 1997). Even with abundant pollinators, however, fruit set and yield can vary significantly between genetically distinct clones due to varying degrees of self-sterility and varying degrees of compatibility among other clones (Free 1993). The influence of clonal patchiness (genetic variability) within a field may have the effect of reducing the incidence of insect pest outbreaks, but this phenomenon has not been thoroughly investigated (DeGomez et al. 1990a).

Lowbush blueberry plants are difficult to establish, and although there are a few commercial cultivars (Augusta, Brunswick, and Chignecto) most commercial plantings are developed in areas where blueberries are already established naturally. This is accomplished by clearing the forest and using herbicides and/or fire to minimize interspecific plant competition. Lowbush blueberries are produced on a wide range of soil types, but the better producing areas tend to be characterized by well-drained sandy loam with a surface layer of organic matter. Growth is optimal in soils with a low pH (pH of 4.6–5.2 [Chiasson and Argall 1996]). Production is best where adequate snow cover occurs, protecting the flower buds from winter kill and desiccation. Growth is best in cool climates with adequate amounts of rainfall during midsummer (2.5 cm/week [Chiasson and Argall 1996]) although overhead irrigation is becoming more commonly used in Maine and Canada by the larger, more capital-intensive growers (Yarborough 1997).

Production methods of lowbush blueberry can have effects on insect pest population dynamics and the dynamics of associated biocontrol organisms. In addition to insecticide applications, pruning is a cultural practice that may have a significant effect on insect pest populations (DeGomez et al. 1990b; Collins and Forsythe 1995) and entomopathogens (Drummond unpublished data). Growth in lowbush blueberry is determinant, being completed with the death of the apical meristem each year. As the apical meristem dies, perennating buds form in the axils of the leaves. This results in a more bushy morphology with berries being produced lower on the plant over time. Harvesting the crop (raking) becomes more difficult and recovered yield decreases as the plant becomes more branched. Because of this, most growers in Maine and eastern Canada manage lowbush blueberries on a two-year cycle (DeGomez 1988; Chiasson and Argall 1996).

Pruning lowbush blueberries involves flail mowing or burning the top growth, leaving the rhizomes intact. Burning has been shown to be a good control for some insect pests of lowbush blueberry, but it can be detrimental to the maintenance of soil organic matter and possibly entomopathogens residing in the top centimeter of soil. Burning is also five to ten times more expensive than mowing (DeGomez 1988). Pruning is performed either in the late summer or early fall after harvest, or in the early spring of the following year. The year immediately following pruning results in vegetative growth and flower bud development. The second year after pruning is the crop year. Bloom starts in early spring (early to mid-May) and lasts for three to five weeks. The long bloom period is

due to the phenological variation between clones. This presents a challenge for insect pest management because in some years bloom overlaps with pest incidence making it difficult to control pests without killing bees. Fruit develops from early to mid-June, with harvest usually beginning in early August.

Most growers divide their fields into each of the two crop cycles (i.e., half in vegetative and half in cropping for a given year). A few growers manage the entire field in the same manner so that the field is harvested only every other year. The spatial arrangement of cropping patterns due to pruning can be very important to the population dynamics of insect pests that are dependent upon the fruit as a food resource, such as the blueberry maggot, *Rhagoletis mendax* Curran. Fields that are split, in regards to vegetative and cropping cycles, allow the pest to easily move back and forth between parts of the field. Fields that are managed as a single unit force the pest to leave a vegetative field and colonize another cropping field during the season. Conceivably, a specialist entomopathogen that has to track its insect pest host might also be detrimentally affected in fields that are managed as a single unit.

INSECT PESTS

The insect pests of lowbush blueberry are mostly foliage feeders or leaf mass consumers (Pedigo 1989). Phipps (1930) listed 292 species of insects that feed on lowbush blueberry. There are fewer than a dozen, however, that occur frequently enough to be economically damaging and that are considered common pests: the blueberry spanworm, *Itame argillacearia* (Packard); the blueberry leaf beetle, *Pyrrhalta vaccinii* (Fall.); the blueberry flea beetle, *Altica sylvia* Malloch; the blueberry case beetle, *Neochlamisus cribripennis* (Leconte); the strawberry rootworm, *Paria fragariae* Wilcox; the blueberry sawfly complex, *Neopareophora litura* (Klug) and *Pristiphora cincta* Newman; and the grasshopper complex. Phipps (1930) lists ten grasshopper species associated with lowbush blueberry: the two-striped grasshopper, *Melanopus bivittatus* (Say*);* Keeler's spur-throat grasshopper, *M. keeleri luridus* (Dodge); the migratory grasshopper, *M. sanguinipes* (Fabr.); the huckleberry spur-throat grasshopper, *M. fasciatus* (Walker); the clear-winged grasshopper, *Camnula pellucida* (Scudder); the crested pigmy locust, *Nomotettix cristatus cristatus* (Scudder); the sprinkled locust, *Chloealtis conspersa* Harr.; the Carolina grasshopper, *Dissosteira carolina* (L.); the wrinkled locust, *Hippiscus apiculatus* Harr.; and the meadow slant-faced locust, *Chorthippus curtipennis* (Harr.). All

of these grasshoppers are leaf chewers, although occasionally grasshoppers will feed directly on the fruit (Collins et al. 1995a). The blueberry thrips complex, *Frankliniella vaccinii* Morgan, *Catinathrips vaccinophilus* (Hood) and *C. kainos* O'Neill, reside in leaf galls or curls. A leaf tier, the red-striped fireworm, *Aroga trialbamaculella* Cham., does not cause economic yield loss to blueberry, but is a nuisance pest interfering with the raking of berries. The major pest of blueberry, the blueberry maggot, *R. mendax,* infests the fruit and can result in total rejection of a crop by a processor if the infestation is greater than 4.0 maggots per quart of berries (Brown and Ismail 1981).

Fruit Pests and Their Control

Pests

Blueberry maggot flies. Blueberry maggot flies attack lowbush and highbush blueberry, as well as huckleberry, *Gaylussacia baccata* (Collins et al. 1987) and shad bush, *Amelanchier spicata* (Phipps 1930). They overwinter as pupae in the soil, 2-5 cm below the soil surface. Adult emergence is temperature dependent and begins most typically in central Maine in early July continuing through most of the summer until mid-August (Drummond and Collins 1997b). Peak emergence usually occurs by mid- to late July. Adult females will mate and feed for seven to ten days as their ovaries develop to maturity. If emerging in a vegetative field, male and female flies will disperse in search of crop fields containing fruit. Field margins are the first to be colonized, and as the season progresses, flies disperse into the field interior (Drummond 1998). Flies tend to be found in aggregations especially where weeds offer shade and protection from desiccation (Drummond 1998). Flies live for about 30 days in the field (Dill 1987). Upon sexual maturity, female flies seek out ripe fruit for oviposition. Each female may lay up to 100 eggs in a period of 15 to 25 days. Because of an oviposition deterring pheromone, fruits usually receive only one egg. In seven to ten days the egg hatches, and the first instar maggot begins feeding. The first maggots appear in the berries in mid-July. After about three weeks, the larvae complete development through three instars, exit the berry, and drop onto the soil surface. The larvae burrow into the soil to pupate. In Maine, approximately 85% of the overwintering population emerge as adults the following summer. Ten percent of the population spend another year in the pupal stage and emerge the second year, and approximately 5% don't emerge until the third or fourth year (Dill 1987).

The major natural enemies of the blueberry maggot are two braconid wasp parasitoids, *Opius ferrugineus* Gahan and *O. melleus* Gahan, which emerge from the pupae about 25 to 30 days after adult fly emergence (Drummond and Collins 1997b). The parasitoids locate maggot-infested fruit, ovipoisit through the fruit into the maggots, and then overwinter in the blueberry maggot puparium. Predation has also been considered a key factor in regulation of blueberry maggot populations (Boller and Prokopy 1976). Ants, staphylinid beetles, carabids, cecidomyiids, and crickets have been observed preying upon other fruit fly species within the family Tephritidae. Fungi, bacteria, and viruses have been found to infect various tephritid species (Bashiruddin et al. 1988), but to date, none has been isolated from natural populations of blueberry maggot (Sivinski 1996).

Pathogens

Very little efficacy testing of pathogenic agents has been conducted with the blueberry maggot. The following discussion summarizes what natural enemies have been recovered from blueberry maggot field populations, and what organisms have potential for biological control.

Entomopathogenic nematodes. Naturally occurring entomopathogenic nematodes do not appear to be important in regulating the population dynamics of the blueberry maggot (Debouzie 1989). No entomopathogenic nematode species have been isolated from the blueberry maggot, and no efficacy tests have been conducted to assess their potential as yet. However, bioassays and field tests with other tephritid fruit fly pests suggest that they may have potential for biocontrol. *Steinernema carpocapsae* and *S. feltiae* have been used to control the Mediterranean fruit fly, *Ceratitis capitata*, the oriental fruit fly, *Daucus dorsalis,* and the melon fly, *D. cucurbitae,* in the field, with mortality rates of 92%, 85%, and 86%, respectively (Lindegren 1990; Lindegren et al. 1990). The tests showed that 85% mortality could be expected with as few as 500 nematodes/cm^2 of soil. These tests were soil drenches directed at larvae burrowing down into the soil prior to pupation. Pupae are probably not susceptible because of the limited sites for nematode entry into the host (Klein 1990).

Beauveria bassiana (Bals.) Vuill. (Bb). There are no reports of *B. bassiana* infecting the blueberry maggot, but in the laboratory, adult flies have been found to be susceptible (Drummond unpublished data). Strongman et al. (1997) found that in blueberry fields

in eastern Canada nine species of entomopathogenic fungi were present, and that *B. bassiana* was responsible for about 10% of the total insects killed by fungi. Other entomopathogenic fungi found were *Entomophaga aulicae* (Reich.) Humber, *Hirsutella nodulosa* Petch, *Hisutella* sp., *Paecilomyces farinosus* (Holm ex S.F. Gray) Brown and Smith, *Paecilomyces fumosoroseus* (Wize) Brown and Smith, *Tolypocladium niveum* (Rostr.) Bisset, *Verticillium lecanii* (Zimm.) Viegas, and an undetermined fungal species. *B. bassiana* has also been found to be endemic in blueberry soils in Maine (Drummond unpublished data). At present, no studies have been conducted to assess the susceptibility of adult or larval blueberry maggots to *B. bassiana* infection. If any of these stages are susceptible, it is fortuitous that there is currently a strain of *B. bassiana* that is commercially available (Mycotech Corp., Butte, MT). Persistence on the foliage and within the blueberry canopy will be an important consideration for targeting adults because of the adults inactive nature, resting during much of the day and foraging in early mornings and evenings (Phipps 1930:133). This might be overcome if attractive baits, such as found in SureDye® used against Tephritid fruit flies (DeQuattro 1998), can be used in conjunction with an inoculation chamber (Jackson et al. 1992; Furlong et al. 1995). Targeting larvae will require soil drench applications probably during mid- to late summer. The ability to deliver the inoculum through the thick plant canopy to the soil will be one of the challenges that determines whether this fungus could be used on a commercial scale.

Metabolic by-products of Saccharopolyspora spinosa. The metabolic by-products of *S. spinosa* are not pathogens, but they are sometimes considered microbial controls. Although, these compounds have never been recovered from any naturally occurring blueberry insect pest, they may have potential for control of the blueberry maggot. The stage most likely to be susceptible is the adult stage. Efficacy tests have not been conducted with *R. mendax*, but laboratory and field trials directed against the apple maggot, *R. pomonella*, show promise (Moreno unpublished data). It has been shown that the by-products of *S. spinosa*, formulated as Spinosad™ 2SC, can be incorporated into feeding baits to effectively control the Mediterranean fruit fly (DeQuattro 1998). This strategy may reduce the detrimental effect of Spinosad® on nontarget beneficial insects such as *Opius ferrugineus* and *O. melleus*.

Leaf Pests and Their Control

Pests

Blueberry spanworm. Blueberry spanworm is a specialist herbivore of lowbush blueberry. It was first reported on blueberry as a pest in 1896 in New Hampshire (Slingerland 1897), but was not a common pest in Maine until 1980 (Forsythe and Flanders 1982). *I. argillacearia* overwinters in the egg stage on the surface of the soil. Egg hatch occurs in early spring, about the time of lowbush blueberry bud break. Larvae develop through four instars. During the day larvae tend to find shelter at the base of the plant and in the litter. They move onto the plants at night and feed. Early in the season, larvae damage the berry crop by feeding on flower buds and blossoms. Later in the season, the caterpillars feed mainly upon developing leaves. In some years the incidence of caterpillars overlaps with peak bloom, which makes it a difficult pest to control without killing bees. Caterpillars move into the leaf litter to pupate by late June to early July. Moths begin to emerge about two weeks later. Moths are present in the field, laying eggs singly in the litter until late July. There is only one generation per year.

Natural enemies of the spanworm include two undetermined virus morphs (Forsythe and Flanders 1982), *B. bassiana* (Drummond, unpublished data), one species of hymenopteran parasitoid (*Cingilia catenaria*) and three tachinid parasitoid species (*Winthemia quadripustulata, Madremyia saundersii, Zenillia vulgaris* [Wood 1918]). Periods between widespread regional outbreaks of this geometrid can be long, 37 to 38 years in New Hampshire and eight to ten years in Maine, although in most years there are high-density populations in some locations in Maine (Forsythe and Flanders 1982).

Blueberry flea beetle. The blueberry flea beetle is the most common defoliating insect pest of lowbush blueberry. Its only recorded host plants are the lowbush blueberry complex (Phipps 1930). Winter is passed in the egg stage in the leaf litter of blueberry fields. In Massachusetts and Maine, however, adults have been found in early June, suggesting that it might also be capable of overwintering as an adult (Shaw et al. 1950; Drummond unpublished data). Egg hatch coincides with leaf buds opening, ca. mid-May. The larvae climb to the foliage and begin feeding upon the leaf margins. The larvae develop through three instars, finish feeding by late June, and move into the leaf litter to pupate. Adults emerge after completing about two weeks in the pupal stage. Adults feed on the foliage

and lay eggs in July and August. Up to two hundred eggs per female may be laid singly in the leaf litter (Woods 1918). There is only one generation per year. No parasitoids have been found parasitizing the blueberry flea beetle. *B. bassiana* has been found to infect larvae naturally in the field in Maine (Drummond, unpublished data).

Blueberry leaf beetle. Unlike the blueberry flea beetle, the blueberry leaf beetle overwinters only in the adult stage, congregating in the forest border surrounding blueberry fields. In late April, the adults fly into blueberry field edges, mate, and begin to feed on expanding leaf buds. Adults feed on the lower side of the leaves, but not the veins, thereby skeletonizing the leaves. Eggs are placed individually at the base of blueberry stems from late May to late June. Females deposit about 25 eggs over their lifespan (Woods 1915). On average, eggs take about 16 days to hatch. The larvae develop through three instars while feeding upon blueberry leaves and take about 50 days to complete larval development (Phipps 1930). The larvae crawl into the litter to pupate, and a new generation of adults emerges in late July. These adults feed on blueberry foliage well into the fall (mid- to late September in Maine) before seeking forest borders for overwintering. Woods (1915) and Phipps (1930) both have reported the blueberry leaf beetle feeding upon willow in addition to blueberry. No predators or parasitoids have been reported for this insect, but Woods (1915) reported that the entomopathogenic fungus *Sporotrichum globuliferum* Speg. caused epizootics in natural populations.

Blueberry case beetle. The blueberry case beetle occurs wherever lowbush blueberry is native. It feeds exclusively on lowbush blueberry. The adults overwinter in the leaf litter in blueberry fields. They emerge from overwintering between late April and early May. Egg laying begins in early May and peaks by mid-June. Eggs are laid singly, attached to the blueberry stem by a short stalk. To protect the egg, the female beetle covers it with a black bell-shaped case of excreta. After ten to 14 days, the egg hatches and the larvae chew a hole through the case, which breaks free from the stalk, but continues to serve as protection for the larvae throughout their development. The larvae feed for about three weeks, adding to the case with their own excreta. Pupation occurs upon the blueberry plant within the case. The adult beetles emerge from their cases in late July or early August and feed upon blueberry stems until fall, when they move into the litter to overwinter. Damage in the late summer and fall is often the most serious since feeding often results in girdling and death of the stem (Canada Dept. of Agric. 1994).

Factors affecting the population dynamics of the blueberry case beetle have not been studied.

Strawberry rootworm. Strawberry rootworm is mainly a pest of strawberry (*Fragaria* spp.) in northeastern U.S. and Canada, but the adults can be injurious to lowbush blueberry. The adults overwinter in the leaf litter in blueberry fields, or in the surrounding forest floor. They become active and begin to feed in early May. The adults are often not seen because they are active at night. Eggs are laid on the soil at this time, and the larvae, after hatching from the eggs, burrow into the ground where they feed on the roots of strawberries and raspberries (*Rubus* spp.) The larvae are not a pest problem on blueberries. The new adults that emerge between mid-July and late August feed on blueberry foliage for the remainder of the summer. There is only one generation per year.

Blueberry sawfly. The blueberry sawfly overwinters as a pupa in a cocoon and emerges in early to mid-May (in eastern Maine). They lay eggs in newly developing lowbush blueberry leaf whorls. The egg hatches in one to two weeks (Collins et al. 1994a). Larvae feed inside the leaf whorl, killing the leaves and filling the whorl with feces. Older larvae appear on the surface of the developed blueberry foliage in late May and early June. Larvae continue to feed until mid- to late June before spinning cocoons in the litter where they overwinter. One generation occurs per year. Blueberry sawfly has not been extensively studied, and no natural enemies have been reported in the literature.

Grasshoppers. Grasshoppers vary in abundance and species diversity both temporally and geographically throughout the lowbush blueberry production region. Of the 37 acridid species found in Maine (Morse 1921), ten species are associated with blueberry and five species are common: the two-striped grasshopper, Keeler's spur-throat grasshopper, the migratory grasshopper, the huckleberry spur-throat grasshopper, and the clear-winged grasshopper (Collins et al. 1987). Grasshoppers associated with lowbush blueberry are generalists and feed on many plant species. Both nymphs and adults feed on foliage and chew and bite the berries. Feeding damage on the berries is often detected as a callused scar on the fruit. Most grasshoppers overwinter in the egg stage in the soil. Eggs are usually placed 2–5 cm below the surface of the ground along roadsides, edges of blueberry fields, and in open areas of managed fields. Eggs begin to hatch between May and June depending upon the species. Nymphs emerge from the soil and feed on the nearest

vegetation. Within four to seven weeks, depending upon species and temperature, the nymphs undergo several molts and metamorphose into adults. Egg laying may begin as early as the middle of July and continues through September. Eggs are usually laid in pods of ten to 100 eggs. The common grasshopper species in Maine and eastern Canada have one generation. There are many natural enemies of grasshoppers, ranging from birds, skunks, other grasshoppers, hymenopteran parasitoids, entomopathogenic nematodes, protozoans, and fungi (Goettel and Johnson 1997).

Red-striped fireworm. Red-striped fireworm has been collected on lowbush blueberry, huckleberry, cranberry, locust, and oak (Phipps 1930). It spends the winter as a full-grown larva within tied blueberry leaves in the litter. In late April and early May, the larvae pupate, and new adults emerge in one to two weeks (Collins et al. 1994b). Adults can be seen in the field as early as mid-May and are usually present until early August. Eggs are laid singly on blueberry leaves in early July. Upon hatching, the larvae web together two or three leaves and feed within the tied leaves. As the larvae grow, more leaves are webbed together to provide food and shelter. In heavily infested fields more than 50% of the blueberry stems may have leaf ties (Collins et al. 1994b). In late August and September, larvae terminate feeding and crawl onto the ground where they will spend the winter. There is only one generation of red-striped fireworm per year. Factors regulating the population dynamics of red-striped fireworm have not been reported in the literature.

Blueberry thrips. Blueberry thrips is a pest complex comprised of the three thrips species *Frankliniella vaccinii, Catinathrips vaccinophilus,* and *C. kainos*. *F. vaccinii* was first observed on lowbush blueberries in Maine in 1926 (Phipps 1930), and *C. kainos* was first reported in eastern Canada in 1947 (Wood 1960). *F. vaccinii* and *C. kainos* are found in eastern Canada, Maine, Massachusetts, and New Hampshire, while *C. vaccinophilus* is found in New York and Pennsylvania (Langille and Forsythe 1972). Host plants for blueberry thrips are the lowbush blueberry, sweet fern, and whorled loosestrife, although lowbush blueberry appears to be the preferred host (Wood 1953). Thrips are more prevalent on *V. angustifolium* compared to *V. myrtilloides*, possibly due to the pubescent foliage of *V. myrtilloides,* which might hinder movement and feeding (Wood 1953). Both species overwinter in the soil (7–10 cm in depth) as adult females (Langille and Forsythe 1972). They emerge from the soil in late May to early June. Feeding by the adults causes the newly unfolding leaves to curl and thus form a protective

layer over the adult thrips. Eggs are laid in the developing leaf tissue and immature thrips hatching from the eggs are found in the leaf curls until late June to mid-July (Collins et al. 1995b). High densities of 100 immatures per leaf curl and 100% of the stems containing a leaf curl can result (Langille and Forsythe 1972). The immatures increase in numbers until about mid- to late July, after which adults become increasingly abundant. The time interval from egg to adult is about 29 to 30 days in the field (Wood 1953). In mid-August the thrips mature into adults, disperse a short distance by flight and then burrow back into the soil for overwintering (Lathrop 1942). There is one species of mite (species undetermined) belonging to the family Laelaptidae that is predatory upon blueberry thrips inside the leaf curls (Wood 1953). No diseases or parasitoids have been found attacking blueberry thrips, although there are a number of insect species (cecidomyiids, aphids, coccids, and tortricids) that use the thrips leaf curls as feeding habitats, possibly competing with them for food (Wood 1953).

Pathogens

Research on biological control of blueberry insect leaf pests has been conducted over the past 15 years. Much of this research has focused on the efficacy of the bacterial toxin derived from *Bacillus thuringiensis kuristaki* for control of the blueberry spanworm. In the last three years (1996-1998) laboratory and field efficacy tests using entomopathogenic nematodes, *S. spinosa* (Spinosad®), *B. thuringiensis kuristaki,* and the fungal pathogen, *Beauveria bassiana,* have been conducted against a number of the blueberry leaf pests. The following discussion summarizes the results of these studies.

Entomopathogenic nematodes. Commercially available nematodes have only been tested against the blueberry spanworm and the blueberry flea beetle. These tests are preliminary, consisting only of laboratory bioassays (Drummond and Collins 1998a). The results suggest that if nematode survival on the foliage can be maintained long enough for infection, then there might be promise for these biocontrol agents in lowbush blueberry. Blueberry spanworm might be the best candidate for testing because the caterpillars feed most actively in the evening just after sunset and before dawn. Thus, evening applications of entomopathogenic nematodes might provide control. *Steinernema riobravis* applications resulted in limited control of 3rd and 4th instar blueberry flea beetle in the laboratory (40% mortality after five days at twice the comparable recommended field dose of 5 billion infectives/ha or 2 billion infectives/

acre). *S. carpocapsae* applications, formulated as Vector TL™, resulted in 92% mortality of 3rd instar blueberry flea beetles after four days, and 100% mortality after six days at the comparable recommended field dose of 2.5 billion infectives/ha or 1 billion infectives/acre). Third instar blueberry spanworm caterpillars are also quite susceptible to *S. carpocapsae* in the laboratory (which had 95% mortality after three days and 100% mortality after five days at the comparable recommended field dose of 2.5 billion infectives/ha or 1 billion infectives/acre). Entomopathogenic nematodes have not been evaluated for control of other lowbush blueberry pest species. *Heterorhabditis bacteriophora* (Cruiser™) has been evaluated for control of the highbush blueberry pest, the oriental beetle, *Exomala orientalis* Waterhouse, but was not found to be effective (Polavarapu et al. 1998).

Metabolic by-products of Saccharpolyspora spinosa. At the time of this writing (1999), only the blueberry spanworm has been evaluated against the metabolic by-products of *S. spinosa* (Drummond unpublished data). In the field, a single application of these by-products, formulated as Spinosad™ 2SC (at 0.44 l/ha or 6 fl oz/acre), reduced spanworm populations by 95%. A half rate of Spinosad® (at 0.22 l/ha or 3 fl. oz/acre) applied with a half rate of *B. bassiana* (at 1.2 l/ha or 16 fl oz/acre) reduced blueberry spanworm caterpillar abundance by 97%. The mortality occurred within two days in the field. It appears Spinosad® is a very promising microbial insecticide, although efficacy tests against honey bees and parasitic Hymenoptera are warranted. In highbush blueberry, Spinosad™ 2SC (at 0.44 l/ha or 6 fl oz/acre) has also been found effective against the gypsy moth, *Lymantria dispar* L., and the obliquebanded leafroller, *Choristoneura rosaceana* (Harris). The by-products of *S. spinosa,* formulated as Spintor™ 2SC (at 0.22–0.44 l/ha or 3–6 fl oz/acre), reduced damage significantly in highbush blueberry due to cranberry fruitworm, *Acrobasis vaccinii* Riley and cherry fruitworm, *Grapholita packardi* (Zeller) (Wise and Gut 1998).

Bacillus thuringiensis kuristaki Berliner (Btk). Blueberry spanworm is the only pest of lowbush blueberry for which growers use Btk on a regular basis. Many formulations are registered for use against this pest: Javelin™, Dipel™, Agree™, and Biobit™ (Yarborough and Collins 1997). The smaller the caterpillars, the more susceptible they are to field rates of Btk (Javelin) (Drummond and Collins 1998a). Not all formulations of Btk, however, are equally effective against the blueberry spanworm. A field efficacy test conducted in Maine in 1996 showed that Agree™ 50WP did not

reduce caterpillars below the control densities, whereas Javelin™ WG and Able™ 50WP did (Collins and Drummond 1997). A similar field trial in 1997 showed that Javelin™ WG, Co Bacil™ WDG, and CryMax™ WDG all reduced spanworm abundance to levels significantly lower than that observed over the season in the control plots. In this test no significant reduction in spanworm larval density was observed with the Bt product, Mattch Bioinsecticide™ (Collins and Drummond 1998a).

Bacillus thuringiensis tenebrionis Berliner (Btt). M-trak™, a commercial formulation of Btt has been tested against larval blueberry flea beetle populations both in the field (at a rate of 7.1 l/ha or 3 qts/acre [Collins and Drummond 1997b]) and in the laboratory (at comparable field rates ranging from 0.0007 l/ha to 7.1 l/ha or 0.0003 qts/acre to 3.0 qts/acre [Drummond and Collins 1997a]) with no success. Efficacy of Btt formulations has not been evaluated against adult blueberry flea beetle, blueberry leaf beetle, blueberry case beetle, or strawberry rootworm in blueberries.

Beauveria bassiana (Bals.) Vuill. (Bb). The entomopathogenic fungus *B. bassiana,* formulated as Mycotrol™ES, has been evaluated against the blueberry spanworm, blueberry flea beetle, blueberry leaf beetle, blueberry thrips, red-striped fireworm, and the blueberry grasshopper complex. Mycotrol does not show high potential against the red-striped fireworm in the laboratory. A dose of 6.87×10^{10} conidia/ha or 2.75×10^{10} conidia/acre only resulted in 42% mortality after 12 days (Drummond and Collins 1997a). Blueberry thrips are also not effectively controlled with high dosages of *B. bassiana*. A soil drench at a rate of 18.9 l Mycotrol/ha or 8 qts/acre did not significantly reduce adult thrips emergence or leaf curling, whether it was applied in the fall or spring. By late summer (four or 11 months post-application), however, thrips within leaf curls were found dead from *B. bassiana* mycosis (Drummond unpublished data). Grasshopper nymphs are very susceptible to Mycotrol, as are blueberry leaf beetle adults. Laboratory bioassays showed that 95.8% mortality of grasshopper nymphs occurred by day 8 when sprayed with a comparable field dose of 6.87×10^{10} conidia/ha or 2.75×10^{9} conidia/acre, and 96% mortality of blueberry leaf beetle occurred by day 12 when sprayed with a comparable field dose of 6.87×10^{10} conidia/ha or 2.75×10^{9} conidia/acre (Drummond and Collins 1997a). Blueberry spanworm caterpillars and both adult and larval blueberry flea beetle are very susceptible to Mycotrol under laboratory conditions (Drummond and Collins 1997a). Field trials testing the efficacy of Mycotrol on these two species resulted in good control

Table 1. Key pests of lowbush blueberry, their current and potential microbial control agents (those field tested), application rates, and selected references.

Key Pests	Microbial Agent	Application Rate	References
blueberry spanworm	Btk (Javelin, Dipel Agree, Biobit)	1133.9 g/ha (16 oz/acre), but depends upon formulation and product.	Yarborough and Collins 1997
blueberry spanworm	Mycotrol ES *(B. bassiana)*	2.4 l/ha (32 fl oz/acre)	Collins and Drummond 1998
blueberry flea beetle	Mycotrol ES	2.4 l/ha (32 fl oz/acre)	Collins and Drummond 1998

of flea beetle in two different trials with a single application at a field rate of 2.4 l/ha or 32 fl oz/acre (Collins and Drummond 1998a) and good control of spanworm with one (Collins and Drummond 1998b) or two applications (Drummond, unpublished data) at a field rate of 2.4 l/ha or 32 fl oz/acre for each application.

PROTOCOLS FOR APPLICATION

Crop Characteristics

Lowbush blueberry is not a high-value fruit crop, but relative to other crops its cultivation can result in considerable gross revenue. The value of the crop is difficult to state as an average dollar amount because the value is determined by yield and price. The yield is highly variable from year to year and from location to location (1,000 to 5,000 kgs/ha). Furthermore, the price can be quite volatile (recent prices of processed berries have ranged between $0.66/kg and $1.00/kg; while the fresh market prices can range between $2.50/kg and $4.00/kg and higher). The five-year running average for lowbush blueberry production in Maine is 29.7 million kgs (Yarborough 1998a). Because of the crop's potential high value, frequent and extensive sampling in the fields (crushing fruit when walking), especially when berries are ripening, should be avoided. Low frequency of sampling can lead to impediments in efficacy testing, especially if multiple applications are necessary.

Field size is quite diverse and can range from less than 0.25 ha to greater than 200 ha (0.5-500+ acres). Generally field size is not a consideration when conducting efficacy trials; however, in the case of highly mobile blueberry insect pests such as grasshoppers and blueberry maggot, field size is important. For highly mobile pests, plot size must be increased to ca. 1/8 ha (Collins and Drummond 1998). The distance between plots must be increased, which may mean that small fields may not be appropriate for efficacy trials unless a design is adopted whereby each field becomes a replicate with a single plot within a field. Or a less desirable incomplete block design could be adopted, where some but not all of the treatments are placed within a field (Mead 1992: 150).

Another crop characteristic that can affect the design of efficacy trials is the clonal nature of the lowbush blueberry crop. Lowbush blueberry plants initially establish from a seed. These plants send out underground stems called rhizomes, which periodically send up new stems above the soil surface and roots below. The original plant, with its spreading rhizome system, constitutes a clone, and each clone is genetically different from its neighbor. Clone size is dependent upon the growth rate of the clone and its age (older clones are usually larger), but an average clone covers 75 to 250 square feet (Yarborough 1998b). An acre of wild blueberries may contain 250 to 500 clones. High genetic diversity in the crop might affect insect development rates, survival, or crop response to feeding damage. Because of this, when conducting efficacy tests on pests with low mobility or caged test populations, one should use either microplots (0.5-5.0 m^2) set up across a single clone (either all replicates on one clone or a single replicate per clone as in a randomized complete block design [Zar 1984:222]), or select a plot size that will encompass multiple clones. Use of large plot sizes that include more than one clone may result in increased heterogeneity, necessitating an increased number of replicates per treatment. One should avoid any design where heterogeneity is maximized, as will be the case if individual clones become the plots, where n = the number of experimental units or sample size and the number of clones.

As in most crop production systems, soil fertility, irrigation, soil acidity, and aggregated weed distributions should be considered in the layout of microbial insecticide efficacy trials. Pre-sampling, or the researcher's knowledge, should be used to position statistical blocks for the purpose of partitioning out heterogeneity due to these factors. Ignorance of these factors could result in tests where some treatments experience higher relative humidity or higher soil moisture either due to direct effects, such as irrigation, or indirect

effects, such as greater canopy development due to uneven soil fertility. Differential soil moistures or relative humidities can result in undesirable plot-to-plot differences in entomopathogenic nematode survival (Kaya 1990), Bt toxin activities upon the foliage (Dunkle and Shasha 1989), and survival and germination rates of *B. bassiana* conidia (Benz 1987).

Crop pruning practices (burning vs. mowing) should also be considered before initiating an efficacy trial. If the objective of the biocontrol strategy is to inoculate the soil so that long-term persistence of the microbial agent occurs, then the trial should involve comparisons between both methods of pruning. It is known that burning has a detrimental effect on overwintering inoculum of plant fungal pathogens that infect blueberry, resulting in a reduction in disease incidence following burning compared to mowing (Yarborough 1998b). Therefore, it might be hypothesized that persistence of entomopathogenic microbials would be less successful in burned fields.

As in most small fruits, pollination is a requisite for lowbush blueberry production (Yarborough 1998b). Almost all of the pollinators are insects, and the great majority of these are bees (Hymenoptera: Apidae). Because of the overlap between many insect pests of blueberry and bloom, one of the priorities in developing pest management strategies is to ensure that they do not detrimentally affect native or exotic (such as honey bees and alfalfa leafcutter bees) pollinators, nor the resulting fruit set. Therefore, all efficacy tests aimed at lowbush blueberry insect pests should also be paired with efficacy tests on bee pollinators. It is not practical to test all bee species associated with pollination of lowbush blueberry since there are 40+ species in Maine alone (Stubbs et al. 1993).

Assessment of fruit set and yield in control plots compared to treated plots will allow for conclusions regarding effects of treatment on pollination, but not necessarily on pollinators. A reduction in fruit set or yield, due to fewer ovules fertilized in a berry, can be a result of mortality in pollinators, but may also be due to repellency of treatments to the bees or deleterious crop physiological responses to the treatments (such as increased June drop). While the reason for the reduction can not be assessed by measuring fruit set and yield, it can provide important evidence suggesting whether a deleterious effect occurs as the result of a microbial treatment. To measure fruit set, 30 random blueberry stems within each plot representatively spread across all clones should be marked with a 3–5 cm length of string tied about the base of the stem. This should be performed before bloom, prior to the beginning of May. After tight

cluster has passed, the flower buds should be counted on each marked stem and recorded. With the completion of bloom all viable fruit should be counted on each stem and the percentage of flower buds resulting in fruit can be calculated. In early July, a second count of the viable fruit should be taken since many fruit that were poorly pollinated will drop from the plant. Calculating the percentage of viable fruit at this time will result in an estimate of "percentage fruit" or "percentage yield." Yield estimates can be taken in early August. Measures of both pounds of berries per unit area (pounds/acre) and berry weight (mean weight in grams of 100 individual berries from each plot) should be made. In addition, a subsample of the weighed berries (30 berries per plot) should be taken, and the number of seeds (fertilized ovules) per berry should be counted and recorded. Analysis of variance (ANOVA) or multiple analysis of variance (MANOVA) can be used to detect reductions in any or all of these measures of pollination (Dafni 1992).

To assess possible side effects on the pollinator community of microbials used for insect control, we have developed a field cage bioassay to assess mortality and pollination efficiency of bees foraging on treated blueberries. As it is difficult to collect native bees in the numbers necessary for such tests, we use commercially available bees that are presently used in lowbush blueberry production: the honey bee, *Apis mellifera*; the bumble bee, *Bombus impatiens*; and the alfalfa leafcutter bee, *Megachile rotundata*. Tests with these bee species represent three size classes of native bees (large: bumble bee, medium: honey bee, and small: alfalfa leafcutter bee), thereby, allowing speculations to be made on the susceptibility of the native pollinator community to the application of microbial control agents. Replicated cages (4mx2mx2m), a minimum of three per treatment, are set up prior to bloom (Stubbs et al. 1998). Blueberry stems are marked inside each cage for later assessment of fruit set and percentage yield. Treatments are made inside the cages and in addition, paired treatments are made outside the cages to compare the presence of the pathogen both inside and outside the cages and assess the effect of the cage on pathogen persistence. Bees are usually introduced after the applications since in general it would not be recommended to spray bee colonies directly, even with a "safe" bioinsecticide. Known numbers of bees are introduced into the cages. Honey bees are introduced as nucleus colonies (nukes) since full-size double brood chamber colonies are too large to have sufficient foraging within a field cage. After bloom or the recommended period of time in which all effects from the pathogen are hypothesized to have been expressed (as adult mortality or brood

mortality), adult and immature bee counts and viable fruit per stem counts are made. Calculations of bee mortality and pollination efficiency are compared using analysis of variance or other more appropriate statistical tests (such as ANCOVA or logistic regression).

The results of these cage studies should be interpreted with caution because bees are confined to foraging on a treated crop where they may not be under real field conditions. However, these tests do provide an initial estimate of the deleterious effects that some entomopathogens may have on bees. Laboratory dose-response bioassays can also be used to ascertain general susceptibility of bees, although again caution should be used in these studies, especially with social bees like the honey bee that can regulate the hive temperature, often to levels suboptimal to fungal pathogens (Loesser et al. 1997). Field tests on the effects of entomopathogens on bee communities can be conducted without cages, but this is logistically much more complicated, expensive, and has greater risk to the grower and bee keeper. Because some bee species (honey bees and some species of bumble bee) forage long distances (up to 3–5 km), replicated small plot trials are not appropriate. Trials need to be performed in very large fields where large plots (probably 1–2 ha) can be spaced far apart (at least ½–1 km), or where individual fields and their bee pollinators can be used as the experimental units.

Plot Characteristics

As mentioned in the previous section on crop characteristics, plot size and spacing between plots will be determined in large part by the mobility of the pest. Entomopathogens in blueberry may be evaluated in either small plots (ranging from 1 m^2 [blueberry thrips] to 100 m^2 [most of the leaf pests]), or in large plots (0.1- to 2-ha plots [blueberry maggot and grasshoppers]). The plot size will depend upon the insect pest and the method of application (aerial application requires larger plots than boom or airblast application). Bottomless buckets or cut stove pipe sections (ca. 452 cm^2) pushed 10–15 cm into the soil also may be used to evaluate entomopathogens in blueberry. These microplot rings are excellent for evaluating efficacy of the fungal pathogen *B. bassiana* against blueberry thrips both as a foliar application and as a soil drench. Small field cages (1 m^2) have been used successfully for efficacy trials with the blueberry spanworm and the blueberry flea beetle (Drummond unpublished data). A known density of larvae is placed on pathogen-treated foliage and then cages are placed over the larvae. The advantage of the microplot design (rings or cages) for evaluations is that single

clones can be used for the entire experiment, or at least for individual replications (statistical blocks), thereby reducing heterogeneity of the host plant. Small plots are also well suited for red-striped fireworm because this insect tends to be aggregated in high-density populations covering small areas. Once the eggs hatch and the young caterpillars make their leaf ties, then 15 m^2 plots (with 2 m of buffer distance) can be laid out and treatments can be made with a backpack sprayer. Efficacy of the treatment in this case is based on the number of leaf ties per m^2, compared to the control treatment. In general, microplot trials are suitable for pathogen efficacy against blueberry insect pests. They are not suitable, however, for determining reduction in yield loss due to use of the entomopathogen since microplots are usually much too variable in berry yield to detect differences of 10% to 20% (which are levels at which many economic thresholds are based).

Most of the leaf pests of blueberry are sampled with a sweepnet and therefore plots need to be large enough to sample frequently without destroying the plants or killing larvae. Plots ranging in size from 50 to 100 m^2 (with 2 m buffer between plots) work well for these pests when a backpack sprayer or boom sprayer is used for treatment applications (Collins and Drummond 1997). Again, because of the clonal nature of the blueberry crop, high numbers of replication are necessary and a minimum of five to six replicates should be used with 50- to 100-m^2 plots. Also, rectangular plots are better than square plots for reducing the variation in insect density and berry yields between plots. Pre-spray sampling for pest density can also aid in separating treatment effects from background variation in pest density. The plots can then be randomly assigned to treatments irrespective of the pre-spray counts. Analysis of covariance (ANCOVA) can be used to adjust for the differences in initial pest densities between plots (Gomez and Gomez 1984:424), or the pre-spray counts can be used to select statistical blocks (groups of plots that have similar pest densities) so that initial density is not confounded with final post-spray densities. Yield estimates are best if the entire plots are raked and the berries weighed. Six plots of 100 m^2 generally result in a precision of 20% (percentage of the standard error to mean), a level usually adequate for field trials. If it is not possible to rake the entire plot, then 20 1-m^2 subplots within each 100-m^2 plot will result in a similar level of precision (Yarborough unpublished data).

Tests for blueberry maggot should rely upon large plots of 0.1 to 1+ ha spaced at least 100 m apart due to the insects' extreme mobility. Three to four replicates of each treatment are suitable for

detecting reasonable differences in efficacy (Collins and Drummond 1998). Because blueberry maggot flies colonize a field from the field edge, all plots should be laid out parallel to the field border. Aerial trials require that plots of different treatments be far enough apart to prevent significant drift between plots (>75 m) when applied under optimal environmental conditions. In most cases, aerial application trials require that the field serves as the experimental unit (a single treatment plot per field). Studies focused on comparison of the levels of berry infestation (maggots per quart) require a minimum of ten to 15 quarts of berries sampled from each plot (Collins and Drummond 1998).

Application Methods

A backpack sprayer is suitable for initial field trials of blueberry leaf pests. However, it is a good idea to eventually use application equipment that is similar to that used by growers. Blueberry growers in the northeastern U.S. and Canada rely upon tractor-driven boom sprayers, airblast sprayers, and aerial application by both fixed-wing aircraft and helicopter. Application of entomopathogens could also be made through irrigation systems, but at present this is not being practiced in the Northeast. In fact, for nematode application, injection into irrigation systems may be the most effective application method (Takeyasu 1994). The evaluation of entomopathogens with these different sprayers means that buffer zones will have to be chosen that are appropriate for a given sprayer. Our drift studies (Drummond and Collins 1998b) suggest that in lowbush blueberry a suitable buffer zone for a high-volume boom sprayer is about 10 m under ideal weather conditions and at appropriate nozzle and nozzle height selection (Spray Drift Task Force 1997). For an airblast sprayer the buffer zone should be about 60 m, and for aerial spraying the buffer zone should be about 75 m (Drummond and Collins 1998b).

Another important point regarding sprayer type in entomopathogen-based control strategies is canopy penetration. High-volume or dilute sprayers such as boom sprayers are capable of delivering entomopathogens to the lower strata of the blueberry canopy, based upon their ability to deliver a high density of spray droplets to this region (Drummond and Collins 1998b). Airblast sprayers are a little less efficient than boom sprayers at delivering high densities of spray droplets to the lower canopy. Aerial applications are the least efficient at delivering high densities of spray droplets to the lower blueberry canopy, especially late in the season when applications are aimed at blueberry maggot and the canopy is

relatively dense compared to canopy development in early spring (Drummond and Collins 1998b).

Pattern and Type of Infestation

Based upon those blueberry insect pests that have been studied, one can generalize that they are spatially distributed in aggregations, or patches, within a field (Drummond and Collins 1996; Drummond and Collins 1997b). This is true for all of the pests except perhaps when densities are extremely low and it is not possible to distinguish their pattern from one of randomness throughout the field. This patchiness or clumped distribution is not necessarily the case with respect to their between-field distribution. In other words, if a particular field within a region is infested with a pest, it is not necessarily likely that the neighboring fields will be more infested than a more distant field. This phenomenon of not being aggregated at the between-field spatial scale may not represent a specific spatial characteristic of the pest species, but more likely is a result of varying management practices including insecticide applications. As the data on regional pest densities are lacking for lowbush blueberry production, it is difficult to know for sure. The best data available is that reported for blueberry maggot by Brown and Ismail (1981), which suggest that 80% of blueberry fields are likely to have blueberry maggot and 40% of those will have densities above threshold levels. One factor that has previously been mentioned that affects the between-field distribution of blueberry spanworm and red-striped fireworm is pruning method. Burning kills these two species because they overwinter in the field.

The within-field spatial distribution of blueberry pest insects has important implications in relation to the evaluation of entomopathogens. First, as mentioned in the section on plot characteristics, four to six replicates per treatment combination are needed for most field trials using naturally occurring blueberry insect pest populations. The number of replicates is a direct result of the aggregation or patchiness. Long narrow rectangular plots should be more efficient at reducing the variability between plot densities (Elliot 1977), but spray application equipment may limit one's ability to select plot dimensions.

More detail on the implications of aggregated insect distribution for experimental design and plot layout can be found in Elliot (1977) and Dent (1997). However, insect pest aggregations also have important considerations for pathogen/host dynamics following application. The level of aggregation (distances between patches and density of insects within patches) of the insect pest may have important implications for cycling or horizontal transmission of an

entomopathogen (Anderson and May 1992; Drummond et al. 1996). Additionally, long-term persistence of the pathogen in an agroecosystem, as has been demonstrated in Maine potato production with the use of *B. bassiana* for control of the Colorado potato beetle (Drummond and Groden 1996), may be tightly linked to the insect host spatial and temporal patchiness. This area of research needs more attention because it represents a significant advance in the way we deploy entomopathogens, moving from a bioinsecticide strategy to a more holistic entomopathogen management strategy that relies upon inoculative releases, horizontal transmission, and persistence, as well as inundative bioinsecticide releases. Analytical methods for detecting cycling or horizontal infection of entomopathogens in field populations have been developed by Drummond et al. (1997 [see Appendix]).

General knowledge regarding the cause of, or basis for, insect pest aggregations should also be a top priority for research in blueberry pest management and could have important implications for use of entomopathogens in IPM. Being able to predict where high-density populations of pests occur may allow targeting small areas within fields for treatment instead of entire fields. For instance, blueberry maggot populations usually are aggregated within a field and these aggregations are highly correlated to field edges early in the colonization process and non-blueberry vegetation, especially patches of the weed sweet fern, later in the season during peak oviposition (Drummond and Collins 1997b). Spot applications of entomopathogens in areas of high pest density may foster establishment of the agent and may be a more cost-effective means for introducing these materials into cropping systems.

Application Dates

Application dates of entomopathogens for control of blueberry insect pests, for the most part, depend upon the phenology of the pest's susceptible stage and the efficacy and speed at which the entomopathogen kills the pest. An exception to this would be the use of soil drenches aimed at soil-borne overwintering stages such as adult blueberry thrips and blueberry maggot pupae. Factors affecting application date in these cases would be pruning date, harvest date, and soil temperature and soil moisture. At present only preliminary efficacy trials have been conducted using *B. bassiana* (Mycotech GHA strain) as a soil drench against blueberry thrips adults in the soil both in the fall and spring. Initial trials have not resulted in acceptable levels of control (Drummond unpublished data).

Use of Btk to control blueberry spanworm requires applications aimed at early instar caterpillars in early May. Because egg hatch occurs over a two- to three-week period, a second application should be made four to seven days after the first application if the economic threshold of five larvae per set of ten sweeps in a bearing field, and three larvae per set of ten sweeps in a vegetative field is exceeded (Yarborough and Collins 1999). Applications of Btk directed against 4th instar blueberry spanworm caterpillars in late May to early June are not as effective (60% mortality for 4th instars vs. ca. 90% mortality for 1st through 3rd instars at recommended field rate of 1 lb/acre Javelin™ [Drummond and Collins 1998a]). The metabolic by-products of *S. spinosa,* such as the commercially formulated Spinosad®, on the other hand, are extremely lethal to all blueberry spanworm larval instars (Drummond unpublished data), so timing of application could be delayed until later in the season if defoliation is not too severe. By delaying the application date, a greater proportion of the blueberry spanworm population will have hatched and will be susceptible to the spinosids, which reduces the likelihood of a second application. *B. bassiana* is also lethal to all of the blueberry spanworm caterpillar instars (Drummond and Collins 1997a). The timing of application could be similar to what might be expected with the spinosids, but it is not known how quickly caterpillars cease feeding after they become infected in the field, although time to death ranges from three to five days in the laboratory (Drummond, unpublished data). This aspect of control of blueberry pests with *B. bassiana* is important if the application strategy is designed to minimize crop loss due to feeding and not just maximize caterpillar population density mortality.

Blueberry flea beetle has a similar phenology to blueberry spanworm. At present Spinosad® and *B. bassiana* are the only microbials that have been field tested against this leaf-feeding pest. Because larval phenology is a result of overwintering egg hatch in the spring, optimal timing of applications can be difficult since they depend upon spring temperatures. In cool springs, hatch rate is slow, thereby increasing the period of larval recruitment. If, after initially killing the first few cohorts of larvae, additional recruitment raises the population above the economic threshold (30 to 50 larvae/set of ten sweeps), multiple sprays may be necessary to effectively control populations (Yarborough and Collins 1999). In addition, adults emerge in July and will stay in the field feeding upon blueberry foliage through August. Both Spinosad® and *B. bassiana*, unlike the nematode *S. carpocapsae* (Drummond and

Collins 1998a), kill adult blueberry flea beetles, so a late summer application may be adequate to reduce late summer damage.

The blueberry sawfly and the blueberry leaf beetle are also spring pests and have similar phenologies to the larval stages of the blueberry spanworm and the blueberry flea beetle. The dates for application, depending upon the action of the pathogen, would be between mid-May and early June in most years.

Using *B. bassiana* or any other living and replicating pathogen to control any of these spring leaf-feeding pests should not be considered similar to using a bioinsecticide (see Pattern and Type of Infestation). If the potential for horizontal transmission (spread of microbial disease from an infected individual to a susceptible individual within the same generation) is great, then the time of application could be determined based upon expected subsequent infection levels. In this case, a later initial application might decrease the level of subsequent infection due to horizontal infection.

Timing the application for control of the red-striped fireworm and blueberry thrips is difficult. A narrow window exists between the time these pests are actively searching for foliage to feed on and the time that they become protected by the blueberry leaf tie or the leaf curl stage, respectively. The application window for blueberry thrips is when plants have between 0.5 and 2.5 cm of vegetative growth in the early spring (between mid- to late May and mid-June [Yarborough and Collins 1997]). Soil drenches might extend this period, allowing application of microbial organisms much earlier in the season or the previous fall when adult thrips are still in the soil. The application time frame for red-striped fireworm is early to mid-July (Collins et al. 1994b). Adult moths can be observed flying in the fields during oviposition. Timing could be improved if a degree-day model was developed to predict egg hatch from a biofix such as first moth capture (as is common with the codling moth in apple [Pedigo 1989]). The red-striped fireworm does overwinter as a fully developed larva and pupates in late April. Therefore, an entomopathogen that can infect the larval stage before pupation in the early spring could have potential. In this case, the timing for application could either be in the fall or early spring prior to late April.

The stages of the blueberry maggot most likely targeted for biological control by entomopathogens are the pupa and adult. The pupa is the overwintering stage and resides in the soil from August until early July of the following year. Soil inoculations could be applied in the late summer and fall or spring and early summer. There is no record in the literature, however, of entomopathogens being evaluated against this stage. The flies begin to emerge in early

July and continue to emerge through July and into early August. Applications can be timed in vegetative fields, the fields from which emergence usually takes place, to coincide with the period of sexual maturation (seven to ten days post-emergence). Because of the long emergence period and the relatively short time that flies remain in the vegetative fields, multiple applications of an entomopathogen would probably be needed to satisfactorily control the population if population densities of overwintering maggot flies are high. Economic thresholds set at an average of 10 cumulative flies/trap (Dill 1987) should be used to determine the need for additional applications. Most maggot control programs are targeted at sexually mature flies colonizing crop fields. Because flies can only lay eggs in ripe fruit, applications should not be initiated before 3% to 5% of the fruit is ripe (about mid-July [Yarborough and Collins 1997]), although the date for application should also be based upon the economic threshold. Therefore, most entomopathogens would be applied between early to mid-July and early August. Blueberry maggots can also be found in high densities in winnow piles. Application dates aimed at this source would not be initiated until harvest, which is usually the beginning of August.

The last consideration regarding the timing of entomopathogen application is protection of pollinators. With entomopathogens that infect bees, the following guidelines should be adhered to:

- Do not apply these microbial organisms on honey bee hives.
- Make applications before or after bloom, at night (for microbes or nematodes with short persistence), or when bees are not actively foraging in crop or vegetative fields.

Application of Pathogens

To assure delivery of infective propagules to the host insect pest, it may be advisable to determine the viability of materials before field application. Methods for determining viability of insect parasitic nematodes to be used in blueberry pest management systems are described by Bedding (1990). For *B. bassiana*, a small quantity of dried conidia (ca. 2.5 x 10^7 conidia) or formulated material (Mycotrol) is added to ca. 25 ml. of 0.1% Tween20® (Sigma Chemical Company, St. Louis, MO) solution. This solution is vortexed for ca. 30 sec., and three 0.5-ml aliquots are then each plated onto a petri dish of Saboroud's dextrose agar (SDA) or some other translucent media. The petri dishes are incubated at 24°C for 24 hours after which the percentage of germinated conidia is determined under a

light microscope at 200 to 400X. Observations of 100 to 150 conidia per plate provide a precise estimate of viability (see Goettel and Inglis (1997) for further details on determining viability of conidia.). Our experience with new Mycotrol material has been consistently 98+ % viability, and this level of viability has been maintained throughout the field season for materials stored in the refrigerator. If the viability is less then this, however, the amount of concentrate used can be adjusted to achieve the recommended field rate of conidia/acre.

Bt products and Mycotrol used for field tests have been formulated for mixing in water. If dry conidia of *B. bassiana* are used for efficacy tests, however, it is advisable to mix the conidia with a surfactant such as Silwet L-77 (Loveland Industries Inc., Greeley, CO). A 0.01% Silwet solution is sufficient for dispersion of conidia in the sprayer tank.

With living organisms such as *B. bassiana,* the compatibility of the entomopathogen with other pesticides used in crop production is an issue. It is not recommended that *B. bassiana* be mixed with fungicides; however, we have successfully tank-mixed Mycotrol with the fungicide copper hydroxide and observed no loss in viability of conidia (Jaros-Su 1997). It is advisable to test materials for their compatibility before mixing in the tank. We have experienced poor compatibility with Mycotrol ES and a Bt formulation that had been in storage for over a year. Upon mixing, the conidia clumped and precipitated out of the tank mix solution. Compatibility tests can be conducted by mixing a small quantity of the materials at the concentrations required for recommend field rates. For *B. bassiana,* the solution should be allowed to sit for 1 hour to simulate the time in the tank during application, and then viability can be checked by plating on SDA as described above.

Our efficacy tests have been conducted using a CO_2 pressurized backpack sprayer, a watering can for soil drenches, and a tractor-driven boom sprayer. We have noted settling and hence the need for regular agitation to keep *B. bassiana* conidia in solution when using a backpack sprayer that does not have an automated agitator. As the viability of *B. bassiana* on foliage has been shown to decline rapidly with exposure to sunlight (Galani 1984), applications of *B. bassiana* are best made in the late afternoon or evening to maximize the time for feeding pests to encounter viable conidia. This is particularly the case for blueberry spanworm larvae, which feed in the evening and early morning hours. Late afternoon or evening is also the best time for application of Btk products against this pest. For pathogens such as Bt that infect through the gut and hence are dependent on

feeding, however, whether evening applications are preferable over early morning applications maybe dependent on the nighttime temperatures and the pest's feeding rates at these temperatures.

Sampling and Monitoring

Estimation of blueberry pest insect densities is a requisite for conducting efficacy trials with entomopathogens. It may also be desirable to sample pest cadavers and the entomopathogenic organisms resulting from inoculations of entomopathogens on foliage, fruit, and the soil. The following discussion first summarizes sampling methods developed for the major blueberry insect pests and then describes methods for determining infection rates in the field and persistence of the entomopathogen outside of the host.

Sampling methodologies for estimating abundance and economic thresholds of many of the blueberry insect leaf-feeding pests (blueberry spanworm, blueberry flea beetle, blueberry sawfly, blueberry leaf beetle, grasshoppers, blueberry case beetle, and the strawberry rootworm) are based upon use of the sweepnet (Yarborough and Collins 1997). The sampling procedure in Maine involves a 30-cm-diameter net (Yarborough et al. 1993). One sample is ten sweeps from one side of the body to the other (180 degrees). After ten sweeps, all of the contents in the net are shaken out of the net into a tray or box and the number of each pest species is counted and recorded. No more than one sweep is taken per step and a set of ten sweeps is taken every 30 to 60 m. A minimum of 20 sets of ten sweeps should be taken (more if fields are large, greater than 100 acres). The sampling path across the field should be a "Z" pattern. Efficacy trials in small plots (7m x 7m) can be sampled by taking one set of ten sweeps representatively throughout the plot. After each plot is sampled, all counted insects should be returned to the plots so that the insect abundance in the plot is not diminished by sampling. To avoid cross contamination, after sampling a plot, the sweepnet should be dipped in a 10% Chlorox® solution and allowed to air dry before sampling the next plot, and the investigator's boots should also be wiped down with the Chlorox® disinfectant. Efficacy trials in large plots (0.1–1+ ha) should follow the same protocol, except it is not necessary to return insects collected during sampling back into the plots.

The red-striped fire worm and blueberry thrips are sampled by walking linear transects across a field and staking out infested areas (those with leaf curls or leaf ties). The intensity of infestation is calculated by estimating the area of infestation and then sampling 100 random stems within the infested area and counting the

number of stems with leaf ties or leaf curls. The proportion of infested stems within the infested area can then be calculated.

Sampling for blueberry maggot flies is accomplished by use of baited (ammonium) yellow Pherocon® apple maggot traps (Gaul et al. 1985; Geddes et al. 1989). Dill (1987) describes how traps should be set out in a field. They should be deployed before July 1 at a density of 2.4 traps/ha for small fields (≤ 4 ha) and 1.2 traps/ha for large fields (> 4 ha). Traps should be suspended from stakes with the underside 15 to 25 cm above the tips of fruiting blueberry plants. To be consistent with the development of the economic thresholds, the yellow rectangular traps should be placed in a "V" position, with the yellow sticky surface directed down. Traps should be placed along the perimeter of the field 8 to 10 m from the field edge and especially along those edges adjacent to unmanaged blueberry areas or vegetative fields. Traps should be placed every 100 ft along the perimeter, but a few traps should also be placed within the center of the field. Traps should be replaced every two to three weeks. Because flies aggregate in weedy areas, traps should also be placed near weedy areas in a field or plot. Field trials utilizing large plots (0.5–1+ ha) should deploy traps in the manner described above. Trials comprised of small plots (0.1–0.5 ha) should have one to two traps deployed in each plot. In all cases, sampling should be conducted every three to four days, and all new fly captures should be counted, removed, and taken back to the laboratory for sexing and analysis of sexual maturity (Dill 1987).

Maggot sampling involves either dissection of berries under a stereo dissecting microscope (Dixon and Knowlton 1994), or flotation of berries in a sugar solution (Neilson 1987). The best method for field efficacy trials is boiling a quart of berries in water for 3 to 5 minutes, and once the berries have split open, pouring the boiled contents into a black painted tray and inspecting the bottom of the pan for the white immatures (Neilson 1987). Efficacy trials require ten to 15 quarts of berries per plot sampled by raking individual quarts of berries from ten to 15 locations within the plot (Collins and Drummond 1998). Berries should be raked when 80%–100% of the fruit is ripe, but before fruit starts to drop off the plants and before maggots begin to drop to the ground to pupate (mid- to late August).

One method for evaluation of the effect of entomopathogens after application is to sample the resulting infection rates. An estimate of infection rates can be obtained by collecting a random sample of the pest insects from both the treated and control plots. A minimum sample should be ca. 30 insects per plot. Following late afternoon or evening applications of *B. bassiana,* insects are col-

lected from plots ca. 36 hours after treatment to allow for germination and infection. Sampled insects are surface sterilized by dipping them in a 0.1% Zephrin chloride (Winthrop Laboratories, New York, NY) solution for 5 to 10 seconds, followed by a rinse in distilled water. Then the insects are individually placed in petri dishes with fresh foliage and moist filter paper. Depending upon the instar and pest species, fresh foliage is added every one to three days. We usually hold insects for ten to 14 days following collection, evaluating them daily for mortality. Cadavers are transferred to a clean petri dish and held at 100% RH for seven days at 25ºC, during which time they are observed daily for signs of *B. bassiana* sporulation. The number dying in the treated plots and the number dying in the control plots can be used with Abbott's formula to estimate the mortality due to *B. bassiana* (Collet 1991:106). Point estimates of proportion mortality collected over time in conjunction with population density samples over time can be used to estimate the seasonal impact of an entomopathogen on the pest population using the technique described by Groden et al. (1990 [see Appendix]).

Sampling the pathogens after application verifies that the intended dose is achieved and is useful for tracking the persistence of the inoculum. To sample for *B. bassiana* inoculum on the foliage, 30 blueberry stems with foliage are randomly collected from treated and control plots. Stems from each plot are cut to ca. 2 to 3 cm, and a 1 g sample of the pooled stems from a single plot are added to 30 ml. of 0.1% Tween solution in a test tube. This mixture is vortexed for 2 minutes to wash the conidia from the foliage. Two to three serial dilutions of the supernatent should be made. From each dilution, four 0.5-ml aliquots of solution are each plated on petri dishes with the wheatgerm-based selective media described by Sneh (1991). A known quantity of *B. bassiana* conidia should be added to 10 ml of Tween20 solution and plated on the selective media at the same time to provide a standard for comparison with observed colony formation from the field samples. Petri dishes are then incubated for ten days at 21 to 23°C. After incubation, the dishes are observed at 100x with a dissecting microscope and colony-forming units of *B. bassiana* are counted. We mark a subsample of the observed *B. bassiana* colonies on each plate and incubate the dishes for an additional ten days, until the fungal colonies have fully sporulated. At this time, a small sample from the marked colonies is collected with an insect pin and transferred onto a glass microscope slide with a drop of distilled water and observed at 400X to verify that the counted colonies were indeed *B. bassiana.* Recovery of colony-forming units over time are compared to that from samples

collected immediately following application of treatments to track persistence.

To monitor persistence of inoculum in the soil, we collect ten soil cores from each plot using a 2-cm soil corer inserted to a depth of 10 cm. The ten cores from each plot are pooled and mixed thoroughly. The percentage moisture from each sample is determined gravimetrically by oven drying and reweighing a 5-g sample of the pooled soil from each plot. After this, the equivalent of a 1-g dry weight sample is taken from each of the original pooled soil and added to 9 ml of a 0.1% Tween20 solution in a test tube. The soil solution is vortexed for two minutes, two to three serial dilutions are made, and 0.5 ml aliquots are plated on petri dishes of selective media. Dishes are incubated, and *B. bassiana* recovery is evaluated as described above, for more detail on soil isolations, see Goettel and Ingis (1997).

CONCLUSIONS

There is considerable promise for biological control of insect pests that attack lowbush blueberry. Currently, *Bacillus thuringiensis* and *Beauveria bassiana* are registered for use in lowbush blueberry in Maine. Recent field trials with *Saccharopolyspora spinosa,* formulated as the commercial product SpinTor 2SC, have demonstrated that this pathogen is an excellent candidate for control of blueberry flea beetle and blueberry spanworm (Collins and Drummond 1999). The perennial nature of the lowbush blueberry crop represents a landscape that is conducive to establishment and persistence of natural enemies. In addition, the simple nature of the insect pest complex makes development of biological control strategies more straight forward. We suspect that biological control will be researched with more intensity throughout Maine as urban sprawl begins to encroach upon once rural farms and as once acceptable crop production methods become scrutinized by the citizens of Maine in regards to their impact upon groundwater, human health, and ecosystem function and biodiversity. However, research with biological control agents, as outlined in this report, should not ignore investigations into the possible negative effects that biological controls might have on nontarget species. This last area of research will be the challenge to future biological control researchers.

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APPENDIX

Estimating Infection Rates From Field Population Sampling

Calculation of field-level infection rates appears to be straight forward, the density of a population infected divided by the total insect density (noninfected + infected). However, many pathogens either prolong the existence of the host in the field by slowing down growth or they may decrease the existence of the host in the field by reducing longevity. This death rate relative to the natural development of a healthy noninfected host can complicate estimates of infection rate. If the death rate shortens the length of time that a host is present in the field relative to healthy hosts then a collection of individuals from the field for determination of infection rate will be biased such that estimates of infection rates will be less than the true infection rate. If on the other hand, the infected stage persists in the field for a longer period of time than the noninfected hosts, estimates of infection will be over estimates of the true infection rate. Groden et al. (1990) present a method for estimating infection rates by taking into account the difference in residence times of healthy noninfected hosts and infected hosts. The method relies upon knowledge of the time to death (residence time) of infected hosts. This information usually has to be obtained by conducting controlled laboratory experiments at a range of constant temperatures so that a time to death measured in degree days can be derived. Once this is known, a researcher has to sample an insect population multiple times (at least 5 to 7 sample dates) and for each sample date the number of infected and noninfected individuals must be recorded. Sometimes, if infection can not be immediately determined in the field, samples must be brought back to the laboratory and the insects must be individually held and incubated until disease symptoms are expressed. The data is then plotted. Two curves are plotted. The incidence of infected insects versus degree days and the incidence of noninfected individuals versus degree days are plotted. The area (integral) under the two incidence curves are independently calculated (using a trapezoidal integration technique) and the densities of each population (infected and noninfected) are calculated by dividing these areas by their respective residence time in degree days (for healthy hosts the residence time is the development time in degree days and for infected hosts the residence time is the time to death in degree days). Proportion infection over the sampling interval can now be estimated by dividing the infected density by the sum of the infected density and the noninfected density. In summary,

Proportion infestation = [integral (infected) / time to death] / [(integral (infected) / time to death) + (integral (noninfected) / development time)]

Interested readers should see the paper by Groden et al. (1990) for more details.

Estimating Cycling of a Microbial Pathogen in the Field

Until recently, little interest was focused on the potential of cycling of insect pathogens in the field after inoculation of a host insect pest. This is because most efforts in microbial control have been focused on using microbial as insecticides where immediate kill is the goal. However, one of the advantages of using pathogens and other organisms for biological control is their persistence and reproductive increase in the field. How can we measure the amount of cycling or the number of generations exhibited in a field population? This is not an easy dynamic to estimate. Mathematical models of these dynamics can aid us in interpreting the dynamics. A simple difference equation of a density independent host disease relationship is as follows:

$$N(t+1) = a(t) * N(t) * (1-D(t))^c$$

where:

N(t+1) = density of insects at sampling time t+1
N(t) = density of insects at sampling time t
a(t) = density independent survival rate at time t
1-D(t) = proportion of healthy insects (those avoiding infection)
c = number of disease cycles

Drummond et al. (1998) have shown that the number of cycles can be solved for by rearranging the above formula and using linear multiple regression to estimate a parameter estimate of c as follows:

$$\log [N(t+1) / N(t)] = \log [a(t)] + c * \log[1-D(t)]$$

If density dependence operates in the field then the model is:

$$N(t+1) = a(t) * N(t)^{(1-b)} * (1-D(t))c$$

where (1-b) is the density dependent term and the solutions for the linear regression are as follow:

1) density dependence operating before disease mortality, then cycling = c

2) density dependence operating after disease mortality, then cycling = c / (1-b)
3) density dependence operating simultaneously with disease mortality, then cycling = [c* log(1-b)] / b

To apply one of these models to field data a researcher needs to sample a population at least two different points in time (t and t+1). The sample dates should have an interval of at least the time to death in the insect host. In addition, the researcher needs a field estimate of infection (see above) and a field estimate of total survival (N(t+1) / N(t)). Then with this field data linear regression can be used to estimate cycling.

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