Introduction

Alfalfa, *Medicago sativa* L., comprises over 50 thousand hectares of cropland in Virginia, and is a valuable forage crop. The alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), is an important early-season defoliator of the crop. Of Eurasian origin, *H. postica* was first reported in the eastern U.S. in the mid-1940's. Having arrived in this country without its natural enemies, the alfalfa weevil rapidly became the most destructive pest of alfalfa in the U.S.

In the late 1950’s, the USDA began a large-scale biological control effort to enhance management of *H. postica*. At least seven species of parasitoids were collected from Europe and established in the U.S. These parasitoids, along with a naturally-occurring entomopathogenic fungus have largely eliminated the need for insecticides to control alfalfa weevil in the northeastern U.S.

In Virginia, however, the alfalfa weevil persists as a major pest, despite numerous parasitoid releases. Based on county extension reports, the severity of pest pressure appears to vary with geography in the state. The goal of the research presented in this dissertation was to advance the understanding of alfalfa weevil ecology in Virginia in order to determine why this insect remains a serious pest in the state. There were three objectives of this research:

1. to assess alfalfa weevil pest pressure, larval parasitization levels, and fungal infection levels in each of the major alfalfa-growing areas of Virginia; the Piedmont, Shenandoah Valley, and southwestern region.
2. to compare the population dynamics and mortality factors of alfalfa weevil at distinct geographic locations in the state.
3. to compare alfalfa weevil phenology with its host crop and primary parasitoids at distinct geographic locations in the state.

Chapter 1
Alfalfa weevil literature review

History and significance of the pest

The alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), is one of the most important pests of alfalfa, *Medicago sativa* L., a valuable forage crop in the U.S. Of Eurasian origin, the alfalfa weevil was first reported in the U.S. in Utah in 1904 (Titus 1910). A second *H. postica* invasion into Maryland in the mid-1940's led to the establishment of this insect in the eastern U.S. (Poos and Bissell 1953, Day 1981). The pest was first detected in Virginia in Loudoun County in 1952 (Evans 1959), and by 1962, it had spread to all regions of the state (Woodside et al. 1968). Annual spring feeding damage by *H. postica* was so devastating and loss in returns so severe in the 1960's, that many growers gave up trying to produce alfalfa (Armbrust 1981). In Virginia alone from 1961 to 1967, alfalfa cropland declined from 105,000 to 33,000 ha due to alfalfa weevil pressure (Woodside et al. 1968).

Since the 1970's, however, populations of alfalfa weevil have been reduced considerably in much of the U.S. by a complex of biological control agents, which include several introduced species of parasitoids and an entomopathogenic fungus, *Zoophthora phytonomi* (Arthur) (Zygomycetes: Entomophthorales) (Day 1981, Harcourt and Guppy 1991, Radcliffe and Flanders 1998). Although alfalfa weevil is considered to be under virtually complete biological control in the northeastern U.S. (Kingsley et al. 1993), it persists as a major pest in Virginia, especially in the Piedmont region where insecticide applications are made annually to protect the first crop of alfalfa from early season larval feeding damage.

Life cycle and seasonal biology

The life cycle of *H. postica* can be characterized by short periods of intensive activity and long periods of inactivity. Alfalfa weevil usually has one generation per year; however, a second or partial-second generation also may occur under warm late-season conditions (White et al. 1969, Loan et al. 1983). In the southern U.S. (below 40° latitude), alfalfa weevil oviposition occurs during the fall, winter, and spring (Woodside et al. 1968, Campbell et al. 1975, Whitford and Quisenberry 1990, Stark et al. 1994), whenever temperatures exceed 1.7˚C (Hsieh and Armbrust 1974). The pith of fresh alfalfa stems is the preferred oviposition site (Pass 1967); however, eggs also are deposited in dead alfalfa stems and weeds including, henbit, *Lamium amplexicaule* L., bulbous bluegrass, *Poa bulbosa* L., common chickweed, *Stellaria media* (L.), and shepherdspurse, *Capsella bursa-pastoris* (L.) (Ben Saad and Bishop 1969, Niemczyk and Flessel 1970). Once an oviposition site is chosen, a female alfalfa weevil will chew a hole through the plant stem wall, insert her ovipositor into the hole and deposit a cluster of 9 to 10 yellow eggs (Burbutis et al. 1967, Niemczyk and Flessel 1970). Coles and Day (1977) determined the reproductive potential of alfalfa weevil to be ≈3,650 eggs per female under optimal conditions in the laboratory. The rate of oviposition increases linearly with temperature up to 30°C (LeCato and Pienkowski 1972, Hsieh and Armbrust 1974).

Alfalfa weevil embryogenesis is also temperature-dependent. Hatching requires 156 degree days above a developmental threshold of 9.0˚C (Roberts et al. 1970). Newly-emerged 1st instars begin feeding on the pith inside the stem, then chew their way out of the stem and crawl to terminal leaf buds where they commence feeding on developing leaves. Alfalfa weevils have four larval instars. The threshold temperature for development is 8.9˚C with an average of 148 celsius degree days required to complete larval development (Litsinger and Apple 1973a). This takes approximately 3 weeks under normal spring conditions in Virginia (Evans 1959). Mature larvae molt into a prepupal stage and spin a net-like silk cocoon within the alfalfa canopy or ground litter (Harcourt and Guppy 1975). The immature weevil pupates within the cocoon and emerges as an adult after the accumulation of 77 DD (Hsieh et al. 1974), or approximately 10 days under normal spring conditions in Virginia (Evans 1959). Newly-emerged adults remain in alfalfa fields for 1 to 3 wk (Prokopy and Gyrisco 1965b), or until 1st harvest (Manglitz 1967), then migrate to suitable aestivation sites usually outside of alfalfa fields at the base of fence rows or in the ground litter of surrounding woods (Tysowsky and Dorsey 1970). After finding a suitable site, adults undergo aestival diapause, during which time sexual development is arrested and metabolic activity is slowed (Litsinger and Apple 1973b). Triggered by changes in photoperiod, alfalfa weevil adults become active again in the fall, migrate back to alfalfa fields, attain sexual maturity, and begin oviposition (Langley and Tombes 1968, Meyer 1982).

Damage

The alfalfa weevil is the most important defoliator of alfalfa in the U.S. (Armbrust 1981). Although *H. postica* feeds on other legumes (DeWitt et al. 1969), alfalfa is the preferred host. Preference for alfalfa is so strong that secondary host crops such as clover intermixed with alfalfa will remain relatively free of damage until the alfalfa is nearly destroyed (Campbell et al. 1975). Both larvae and adults consume alfalfa leaf tissue. Third and fourth instars account for most (>90%) of the feeding (Koehler and Pimentel 1973). The most significant feeding injury occurs to the first crop of alfalfa (Liu and Fick 1975); however, under heavy infestations, regrowth of the second crop can be affected by larval and adult feeding on stubble (Fick 1976). Alfalfa weevil feeding injury can result in reductions in shoot length, yield, forage quality, and stand persistence (Berberet and McNew 1986, Wilson and Quisenberry 1986, Summers 1989, Hutchins et al. 1990).

The severity of crop damage is related to pest density and the stage of plant growth at the time of infestation (Koehler and Pimentel 1973, Koehler and Rosenthal 1975, Shade and Hintz 1983). Hintz et al. (1976) found that the earlier the plant was attacked, the greater was the impact of the larval feeding. Once a sufficient plant height is reached, alfalfa can tolerate defoliation, and low population levels of alfalfa weevil (<3 larvae per stem) can even have a positive effect on the crop by stimulating vegetative growth (Mathur and Pienkowski 1967, Hintz et al. 1976, Weaver et al. 1993). Consequently, economic injury levels for alfalfa weevil have been very difficult to calculate, and are largely based on alfalfa stem height at the time of infestation (Koehler and Pimentel 1973, Koehler and Rosenthal 1975, Luna 1986).

Mortality factors

Factors that affect the survival of alfalfa weevil are related to the age classes of the insect and to distinct periods within these age classes. It is necessary, therefore, to separate the discussion of mortality by life stages.

Adult stage. Little is known about the mortality of adult alfalfa weevils during summer aestivation. Because adults lay dormant on the soil surface for up to 4 months (Prokopy and Gyrisco 1965b, Tysowsky and Dorsey 1970), it is likely that many are killed by ground-dwelling predators. A number of invertebrate predator species have been shown to feed on alfalfa weevil adults including several different carabid beetles (Barney and Armbrust 1981, Los 1982).

Overwintering mortality of alfalfa weevil adults has been well studied (Blickenstaff 1967, Helgesen and Cooley 1976, Hilburn 1985). Weevil adults are cold hardy and can survive temperatures as low as -18°C (Armbrust et al. 1969). In addition, adult weevils burrow through litter and soil to insulate themselves against harsh weather (Tysowsky and Dorsey 1970). Thus, with the exception of extreme winter conditions, relatively few alfalfa weevil adults likely die from exposure to low temperatures (Helgesen and Cooley 1976). Adult mortality during the winter months results primarily from natural senescence and disease, and is relatively consistent from year to year and across regions of the U.S. (Helgesen and Cooley 1976, Blickenstaff et al. 1972). The primary pathogen of adult alfalfa weevils is the fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycetes) (Hedlund and Pass 1967). Many of the adult weevils that survive until spring are killed by parasitoids (Abu and Ellis 1976). The two primary parasitoid species of alfalfa weevil adults are *Microctonus aethiopoides* Loan and *M. colesi* Drea (Hymenoptera: Braconidae). *Microctonus aethiopoides*, in particular, is considered to be the most important biological control agent of alfalfa weevil (Radcliffe and Flanders 1998). Parasitization rates of 70 to 90% by this species are not uncommon in the northeastern U.S. (Brunson and Coles 1968, Abu and Ellis 1976). Also, because parasitism by *M. aethiopoides* induces sterility, alfalfa weevil oviposition can be reduced substantially when this parasitoid is present in fields (van Driesche and Gyrisco 1979). Alfalfa weevil adults are also parasitized by *Hyalomyodes triangulifera* (Loew) (Diptera: Tachinidae), an endemic generalist parasitoid of adult beetles (Thompson 1954). Parasitization by this species, however, rarely exceeds 10% (Miller et al. 1972, Hilburn 1985).

Egg stage. Overwintering alfalfa weevil eggs can suffer mortality from exposure to low temperatures. Morrison and Pass (1974) determined a supercooling point of -23˚C for alfalfa weevil eggs. Eggs can also suffer mortality from prolonged exposure to temperatures that are above the supercooling point, but below the developmental threshold of 9˚C (Litsinger and Apple 1973a). Cothran and Gyrisco (1966) showed that the energy reserves of alfalfa weevil eggs are limited and that, after an initial period of ≈ 90 days, egg survival declines at a rate of 0.7% per day. Also, because weevil eggs are very susceptible to low humidity, their survival is reduced from freeze drying of alfalfa stems during periods of high winds and subfreezing temperatures (Dively 1970, Reid et al. 1989). In the northern U.S., most alfalfa weevil eggs deposited during the fall and winter months die before spring hatch (Townsend and Yendol 1968, Blickenstaff et al. 1972). In the southern U.S., fall and winter-laid eggs contribute significantly to spring larval populations (Woodside et al. 1968, Campbell et al. 1975, Whitford and Quisenberry 1990, Stark et al. 1994). Overwintering egg survival, however, is variable and dependent on temperature conditions and timing of oviposition (Shade and Hintz 1983, Stark et al. 1994).

Relative to overwintering eggs, spring-laid alfalfa weevil eggs suffer very little mortality (Harcourt et al. 1977). Because eggs are generally deposited within green alfalfa stems, this usually protects them from most mortality factors. Nonetheless, some mortality occurs from natural infertility, predation, and parasitism. Two predator species that attack alfalfa weevil eggs are the eastern flower thrips, *Frankliniella tritici* (Fitch) (Thysanoptera: Thripidae), a common plant pest and opportunistic egg predator (Barney et al. 1979), and *Peridesmia discus* (Walker) (Hymenoptera: Pteromalidae), which was introduced from Europe as a biological control agent of alfalfa weevil (Bryan et al. 1993). Predation of alfalfa weevil eggs has averaged only 7.1% thus far in locations where *P. discus* has been established (Dysart 1988). Alfalfa weevil eggs are also attacked by two parasitoid species, *Anaphes luna* (Girault) (Hymenoptera: Mymaridae) and *Fidiobia rugosifrons* Crawford (Hymenoptera: Platygastridae) (Niemczyk & Flessel 1970, Ellis 1973, Hogg and Kingsley 1983). Their value as biological control agents, however, appears to be negligible (Streams and Feuster 1966, Radcliffe and Flanders 1998).

Larval stage. Newly-eclosed larvae may suffer a considerable amount of mortality while attempting to establish on alfalfa growth terminals (Harcourt et al. 1977, Latheef et al. 1979, DeGooyer et al. 1995). Larval establishment is affected by a number of factors including, the severity and duration of cold temperature episodes that the developing eggs have endured, heavy rainfall, desiccation, predation, and disease (Harcourt et al. 1977, Shade and Hintz 1983). A wide variety of arthropod predators of alfalfa weevil larvae have been identified and are reviewed by Howell and Pienkowski (1970), Barney and Armbrust (1981), and Quayogode and Davis (1981). The most important pathogen of alfalfa weevil larvae is the entomopathogenic fungus, *Z. phytonomi*, (Harcourt et al. 1977, Los and Allen 1983), which has been shown to cause severe (>90%) mortality of alfalfa weevil larvae when environmental conditions are suitable (Harcourt et al. 1990). Certain microclimatic factors appear to dictate the magnitude and duration of *Z. phytonomi* epizootics. The onset of the disease is correlated with degree-day accumulations (200 to 290 DD after 1 January), and that, once initiated, epizootic phenology is primarily controlled by atmospheric moisture levels, and terminated when the host population density falls below a threshold of 1.7 larvae per stem (Nordin et al. 1983, Goh et al. 1989).

Alfalfa weevil larvae also are attacked by a complex of parasitoids, including, *Bathyplectes anurus* (Thomson) (Hymenoptera: Ichneumonidae), *B. curculionis* (Thomson), and *Oomyzus* (=*Tetrastichus*) *incertus* (Ratzburg) (Hymenoptera: Eulophidae), which were all introduced into the U.S. from Europe by the USDA for control of alfalfa weevil (Dysart and Day 1976, Bryan et al. 1993). The dominant larval parasitoid in most of the eastern U.S. is *B. anurus* (Radcliffe and Flanders 1998). From 1960 to 1988, more than 15 million specimens of *B. anurus* were released and distributed in the U.S., more than all other parasitoid species of alfalfa weevil combined (Bryan et al. 1993, Kingsley et al. 1993). Research has shown that *B. anurus* out-competes other larval parasitoids such as *B. curculionis* because of its high reproductive capacity, rapid search and handling, and aggressive behavior (Yeargan and Latheef 1977, Harcourt 1990). Also, unlike *B. curculionis*, the eggs of *B. anurus* are not subject to encapsulation by the host larvae (Puttler 1967, Maund and Hsiao 1991). In addition, *B. anurus* reduces competition with the fungus *Z. phytonomi* by laying eggs in older weevil larvae that have a greater probability of escaping death from disease caused by this pathogen (Harcourt 1990). Most biological control researchers consider *B. anurus* second only to *M. aethiopoides* in efficacy of control against alfalfa weevil (Radcliffe and Flanders 1998). *Bathyplectes curculionis* appears to be more important in the western U.S. (Davis 1974, Eklund and Simpson 1977). *Oomyzus incertus* primarily attacks larvae later in the summer rather than at peak larval occurrence in early spring and consequently has relatively little impact on alfalfa weevil population dynamics (Horn 1971, Blickenstaff et al. 1972, Smilowitz et al. 1972).

Pest Management

In the northeastern and north central U.S., biological control generally keeps alfalfa weevil populations below damaging levels (Day 1981, Kingsley et al. 1993). In the southern U.S. (below 40° latitude) alfalfa weevil populations frequently exceed the economic threshold (Campbell et al. 1975, Luna 1986, Berberet and McNew 1986, Copely and Grant 1998). Alfalfa growers commonly apply an insecticide in the spring when field scouting indicates that the larval population will reach the economic injury level, or when alfalfa weevil feeding damage first appears (Luna 1986). Two common sampling methods used for pest management decision-making are the sweep net and shake-bucket techniques. These sampling methods are discussed in detail by Legg et al. (1985), Higgins et al. (1991), and Luna and Ravlin (1992).

Insecticides also can be applied in the fall to kill alfalfa weevil adults before they begin oviposition (Roberts et al. 1987). Fall insecticide applications are less disruptive to natural enemies, but are used as a preventive control tactic only because sampling adult weevil populations in the fall is difficult (Guppy and Harcourt 1977) and not always a reliable indicator of potential larval damage in the spring (Schroder and Metterhouse 1980).

Harvesting alfalfa early in the spring can be an effective weevil control tactic if enough growth is present to justify the harvesting process (Cassagrande and Stehr 1973). Harvesting alfalfa early removes alfalfa weevil eggs and larvae, assures high quality hay, and reduces the chance of losing hay to rainy weather later in the season. Harvesting alfalfa early also has been shown to induce epizootics of *Z. phytonomi* by concentrating weevil larvae in warm and highly humid windrows (Brown and Nordin 1986). This control strategy for alfalfa weevil should be practiced sparingly, however, because stand persistence declines from repeated early first harvests (Latheef et al. 1992).

A late-fall harvest, which removes fall regrowth leaving only stubble to overwinter, also can help reduce alfalfa weevil populations (Hilburn 1985). Returning adult weevils are less attracted to such fields and the removal of fall regrowth limits oviposition sites and exposes overwintering stages to harsher conditions. Dowdy et al. (1992) reported that alfalfa weevil egg numbers were reduced by an average of 55% by cutting alfalfa in late fall. Fall harvests can be detrimental to a stand if not timed properly (Reynolds 1971). Alfalfa needs a recovery period of approximately 6 wk in the fall, during which time it grows 20-25 cm and replenishes its root carbohydrate reserves (Mays and Evans 1973). After this period, or the first hard frost of the year, alfalfa can be safely harvested.

Winter grazing can have a similar effect as late-fall harvesting on alfalfa weevil populations. Senst and Berberet (1980) and Dowdy et al. (1992) reported reductions in alfalfa weevil egg populations of 60 and 70%, respectively, from cattle grazing in December and January. Trampling damage to alfalfa plants should be considered with this control tactic. Grazing after the ground is frozen reduces plant damage and soil compaction.

New grazing tolerant alfalfa varieties, such as ‘alfagraze’ (Boutin et al. 1991), have been released recently, which permit intensive spring grazing without adversely affecting stand productivity. Buntin and Boutin (1996) reported that spring grazing reduced alfalfa weevil larval densities 45 to 60% in Georgia. Because alfalfa is usually not grazed, relatively little research has been conducted on the overall effects of grazing on insect populations. This topic is currently being investigated in Oklahoma and Georgia.