ABSTRACT


The tobacco splitworm (Potato tuberworm, *Phthorimaea operculella*), a microlepidopteran with oligophagous larvae that feed on solanaceous plants, is a reemerging concern for tobacco growers. Tobacco (*Nicotiana tabacum*) is an infrequent host of this species and few studies are available on the biology and ecology of this pest in the tobacco agroecosystem. Potato (*Solanum tuberosum*) is the most common host of *P. operculella*, and the larvae both mine leaves and bore in tubers. In tobacco, larvae primarily act as leaf-miners which can cause economic loss. Adult *P. operculella* flights were tracked with pheromone trap captures and in-field damage in tobacco was assessed in North Carolina from 2008 to 2010. *P. operculella* is a multivoltine pest (2-4 generations per field season, and populations dramatically increase during the late season). *P. operculella* larvae are most common in the lower portion of tobacco plants during the later half of the growing season. *P. operculella* larvae perform better on middle and upper stalk tobacco leaves, as measured by development rate and pupal weight, but appear to feed on lower leaves because they are closer to the oviposition site. Flue-cured harvest alters increases the distance from soil to leaf material over very large areas and significantly impedes *P. operculella* establishment. Larvae are capable of locating leaf material from a distant release site but establishment is reduced the greater distance they are required to travel.

by
Monique Joy Rivera

A thesis submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Master of Science

Entomology

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BIOGRAPHY

Monique Joy Rivera was born to Fernando Louis Rivera, Jr. and Pamela Joy Offner on June 23, 1986 in Orlando, Florida. She was raised in Dover, Delaware where she attended Holy Cross Elementary School and Dover High School. She later attended University of Delaware where she earned B.S. degrees in both Entomology and Plant Science. In 2008, she enrolled at North Carolina State University. Under the direction of Dr. Hannah Burrack, she began to develop and answer questions about *Phthoirmaea operculella* in the tobacco agroecosystem. She will continue her studies in entomology at Rutgers University under the direction of Dr. Cesar Rodriguez-Saona and Dr. Albrecht Koppenhöfer.
ACKNOWLEDGEMENTS

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I would also like to thank my family and friends who have helped make this experience fulfilling and fun. Especially, Anna Chapman, Eleanor Spicer-Rice, the wonderful staff at Cup of Joe, and everyone I worked with in the Burrack lab.
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INTRODUCTION

To be submitted to the Journal of Integrated Pest Management

*Phthorimaea operculella* (Zeller) is a small brown moth (approximately 0.95cm long, with an approximately 1.30cm wingspan) whose larvae feed on solanaceous plants. Potato tuber moth is the Entomological Society of America (ESA) accepted common name but when feeding on tobacco, *P. operculella* is referred to as the tobacco splitworm because of eventual splitting of the leaf caused by larval mining. Now found globally wherever solanaceous plants are cultivated, *P. operculella* is thought to be native to South America where its primary host is potato (*Solanum tuberosum*) also originated (Rothschild 1986). In the 1970s, *P. operculella* emerged as a serious pest for potato growers and as a result, its biology and ecology in this system were extensively studied (Fenemore 1980; Rhondon, et al. 2008). In potato, *P. operculella* mines the leaves of the plant but also bores into tubers. Larvae access tubers through soil cracks but more often, proliferate in post-harvest storage or cull piles. In cultivated tobacco (*Nicotiana tabacum*), larvae are able to bore similarly into stalk tissue and leaf mid-ribs but typically act as leaf miners.

Beginning in 2007 and continuing through the present, large populations of *P. operculella* have been observed in southeastern commercial tobacco fields. Prior to this observation, *P. operculella* was considered an occasional pest of early season tobacco, but recently tobacco injury by *P. operculella* has been observed at the end of the growing season and significant losses have been observed in several locations. Phenology has been tracked in southeastern United States and 3-4 generations were observed in the southeastern United 
States (Rivera, *in prep*). The increased incidence of *P. operculella* damage has resulted in additional pesticide use and prompted research into the ecology of *P. operculella* in *N. tabacum*. Increase in *P. operculella* damage may be due to concentration of production into a smaller geographic area following the termination of the tobacco production allotment and price support program administered by the United States Department of Agriculture (NASS 2008). The elimination of the market quota and price support program, also referred to as the tobacco buyout, resulted in a decrease in the number of farms, an increase in acreage per farm, and a concentration of flue cured tobacco farms in the coastal plain of North Carolina (Dolhman et al. 2009).

*Biology and life history*

While the leaf-mining larval stage causes plant damage, host selection is mediated by the oviposition choice of female moths. Adult moths copulate and females oviposit nocturnally. Adult sex can be differentiated microscopically by observing dorsal and ventral characteristics, and field determination of sex requires either a microscope or a 10X hand lens. The female has a black patch of scales in the middle of the wings when closed and a more conical shaped abdominal tip, while males lack dark wing scales and have a broad abdominal tip (Figure 1.1). Flying ability has been disputed in the literature. Two studies (Foot 1979, Fenemore 1988) posit *P. operculella* is a poor flyer, while one study (Foley 1985) showed that the moth was able to fly 5 hours for up to 10 km non-stop, suggesting that moths have sufficient flying stamina for long distance flight. A New Zealand study found *P. operculella* numbers to diminish over a 40 m distance from the field of origin which supports
the importance of alternate hosts in nearby refugia (Cameron et al. 2002). Natal host also impacts adult movement with larvae feeding on suboptimal hosts, such as feral weeds resulting in adults that disperse greater distances (Coll and Yuval 2004). Male moths exhibit different flight patterns when seeking virgin females or pheromone. Male moths did not respond to female pheromone baits placed 70 cm or higher above the ground, indicating that nocturnal mating occurs close to the ground. (Ono and Ito 1988) Flying short distances, close to the ground, may also be a strategy for avoiding nocturnal predators such as bats (Miller and Surlykke, 2001).

Ovipositing female moths respond to plant traits using mechano- and chemoreceptors on the ovipositor (Valencia and Rice 1982). When females moth were presented a range of hosts, including potato, more eggs were laid on potato tubers or potato tuber extract (Fenemore 1980). On potato, a female moth can lay greater than 200 eggs in her lifetime and is more fecund when tubers are available (Moregan and Crumb 1914, Fenemore 1980). Oviposition site differs by host. On potato, eggs are laid on leaves or in the soil near cracks exposing tubers. In tomato (Solanum lycopersicum) and datura (Datura stramonium), eggs are laid on foliage and soil, but significantly more eggs are laid on the soil in both species. In greenhouse grown tobacco, eggs are almost exclusively laid on the soil or leaf litter surrounding the plant (Varela and Bernays 1988, Van Vuuren et al. 1998). Regardless of oviposition site, moths prefer textured surfaces with egg-sized crevices (Traynier 1975; Fenemore 1978). Only extracts from potato tubers have been shown to stimulate oviposition.
Eggs are spherical, less than 0.1 cm, and iridescent white upon oviposition. As the embryo develops, the color darkens from yellow to brown. Larvae molt four times before pupation. The first instar is 0.3 to 0.4 cm and is capable of traveling up at least 74 cm from the site of oviposition to suitable plant material (Rivera, *in prep.*). The head capsule is dark brown-to-black. The neonate body is light brown and shifts to green or pink in later instars. Fourth instar males (approximately 0.95 cm) can be distinguished from female larvae by two yellowish testes, which can be observed through the larval cuticle in the 5th and 6th abdominal segments (Chauhan and Verma 1991). Larvae spend their entire lifetime in a single gallery. After 4th instars cease feeding, the larva drops from the mine and wanders on the soil surface or leaf litter until pupation. Prior to pupation, the larvae create a silk pupal case. Larvae pupate in soil or leaf litter.

If temperatures are stay above freezing through the winter, every stage of *P. operculella* can overwinter and re-colonize crop hosts in the spring. Even so, it is likely that *P. operculella* are employing other weedy hosts in areas surrounding fields to increase their population before dispersing to tobacco or other hosts. Tobacco fields often have weedy borders or are next to wooded areas, which may provide access to alternate hosts. Larvae are oligophagous on solanaceous plants and individual plants from a few additional families. The known host range includes both cultivated and non cultivated plants (Atherton 1936, Das and Raman 1994). Crop hosts in addition to potato and tobacco include eggplant and tomato, and significant non crop hosts include horsenettle (*Solanum carolinense*), groundcherry (*Physalis pruniosa*), and jimsonweed (*Datura stramonium*).
Pupae are approximately 0.85 cm long, and turn golden brown and deepens to black before eclosion. Pupal age can be determined by the eye area color within the translucent brown pupal case which shifts from yellow (approximately 2 days), to red (3 to 5 days old), and finally to black (5 to 7 or more days old) (Chauhan and Verma 1991). Sex can be determined during the pupal stage using the “scar” method. This method uses the longer distance between the genital opening (resembling a small cut) on the 8th and 9th segment to the tip of the pupae on male pupae to separate the two sexes (Rhondon and Xue 2010). Duration of the pupal stage is variable and depends on ambient temperature. Under ideal conditions (30°C ± 5), pupae can eclose in as few as 5 to 6 days (Moregan and Crumb 1914, Broodryk 1971).

Pest status

*Phthorimaea operculella* is present throughout the world, wherever solanaceous plants are found or cultivated. However, most attention awarded to this species is in areas where it is an agricultural pest. The ubiquitous nature of *P. operculella* has demonstrated its ability to adjust to a wide climatic range. Three separate studies over the span of 70 years in Egypt found three different development thresholds for eggs, larvae, and pupae of *P. operculella* populations (Table 1) (Attia and Matter 1939, Gergis 1987, Daoud et al. 1999). The most recent of these studies (Daoud et al. 1999) suggests an increasing tolerance to cold temperatures in all stages but larvae. Briese (1986) found similar adaptability in field-collected insects from 16 different potato-feeding populations in Australia. Female weight and fecundity were higher in insects from warmer regions, which promote continuous
development, but populations from warmer climates also had a higher incidence of parasitism and disease (Briese 1986).

*P. operculella* is not the only gelechiid species which exploits potatoes. A recent study in the Andes mountains in Ecuador where a multi-species tuberworm complex exists, found *P. operculella* to be the least tolerant (compared to *Symmetricchema tangolias* and *Tecia solanivora*) to cold temperatures (Dangles et al., 2008). Reduced competition for resources may be a contributing factor to why infestation in warmer, more moderate climates has been advantageous for *P. operculella*.

*Tobacco production system*

In the United States, tobacco is grown on ca. 141,688 hectares in a concentrated area (in 7 southeastern states, Florida, Georgia, North Carolina, Kentucky, Tennessee, South Carolina, and Virginia (NASS 2008). Tobacco, which is grown during the late spring through summer, needs little management until flowering. Once flower buds appear at the apical point of the plant, removal of the flower stalk is required in a practice called “topping”. This releases apical dominance and results in nutrient transport to leaves instead of reproductive structures. Unwanted lateral buds begin to form on the plant. These buds are referred to as “suckers” and are destroyed with chemical treatments or by manual removal. At maturity, varietal differences require different methods of harvest and curing.

The most common types of tobacco are flue-cured and burley. For both burley and flue-cured, leaf chemistry is manipulated by limiting the plants’ access to nitrogen. The plant
depletes nitrogen from soil and early-applied fertilizer as it grows. Limited nitrogen access causes the leaf to increase in thickness rather than area after topping, which increases yield (Raper and McCants 1967). Visual differences between flue-cured and burley plants are obvious in the field. Burley is more yellow with leaves that point upward, and flue-cured plants are a deeper green with leaves that drape downward. Kentucky produces the largest amount of burley tobacco in the United States, while North Carolina produces the greatest amount of flue-cured tobacco. Flue-cured tobacco plants produce 20 to 25 leaves that are harvested from the bottom up, 3 to 5 times in 2 to 3 week intervals once the plant matures. Leaves are grouped by stalk position and exposed to a heat source in a closed curing barn. The entire burley plant is harvested intact and hung in an open barn for air curing. Both varieties are graded for sale by leaf stalk position and appearance. Higher stalk position leaves are higher in nicotine and more valuable.

A reemerging tobacco pest

Historically, *P. operculella* damage in tobacco occurred early in the growing season, before topping (Figure 1.2) (Atherton 1936), and was often associated with commercial potato fields or home gardens. The southeastern potato crop is planted in late winter and harvested in the late spring while the tobacco crop is planting in the late spring and harvested in fall. Tobacco production is not consistent throughout the southeast but concentrated over smaller regions within states (NASS 2008). *P. operculella* populations can increase rapidly from minor presence in the field to multiple insects per plant throughout the field in these areas (Rivera, *in prep*).
Female *P. operculella* preferentially oviposit on the soil near tobacco, which may result in avoidance of tobacco’s physical and chemical plant defenses including glandular trichomes and leaf surface chemistry (Varela and Bernays 1988, Van Vuuren et al. 1998). While the leaves of other host plants are also trichome dense, tobacco plants have glandular trichomes, some of which have the ability to release exudates that may deter *P. operculella* oviposition (Keene and Wagner, 1985). Glandular trichomes comprise approximately 80% of trichomes found on flue-cured tobacco, the most common cultivated type of tobacco. Individual *P. operculella* experience plant traits in a gradient, as both trichome density and nicotine content increase with higher stalk positions (Dawson 1945; Van Vuuren et al. 1995). Alkaloids are also distributed within individual leaves. The lamina area in the center of the leaf has the least amount of alkaloids relative to midribs, lateral veins, leaf margins and the basal and tip areas of the leaf. Oviposition at the base of the plant forces neonates to seek out the plant and find the best feeding site within the plant.

Production practices and curing can have impacts on continued feeding by *P. operculella* in the late season and post-harvest. Flue-cured harvest changes the architecture of the plant by successively removing leaves, which increases the distance between the oviposition site and available leaf tissue. Moregan and Crumb (1914) noted *P. operculella* presence in tobacco starts in the lowest leaves and moving to the higher leaves only if population density is high. We confirmed this observation through 3 field seasons in North Carolina (Rivera, *in prep*). Depending on the level of infestation, the process of harvesting flue-cured tobacco could result in either infestation removal or promoting larval relocation to higher, more valuable leaves.
The curing process for flue cured and burley tobacco types impacts larval survival differently. Flue curing barns reach temperatures up to 74°C for at least 48 hours during the initial stages of curing, sufficient to kill *P. operculella* larvae. However, in burley curing barns *P. operculella* can feed until the leaf quality and moisture decrease to an unacceptable level. This is problematic, as damage to leaves continues and population levels can be maintained post-harvest, which can further reduce the value of burley leaves and ensure continued damage in coming seasons.

**Sampling and scouting**

Pheromone traps can be used to detect moth presence in an area with a history of *P. operculella* (Figure 1.4) (Trece © (3537-02 physical trap, 3137-25 pheromone cap)). Depending on size and shape of the field, 4 traps should be organized per 15 acres around the field, positioned between the field and each differing surrounding area (i.e. by roadway, woodlot, adjacent crop). Trap bottoms should be collected weekly and pheromone caps replaced monthly. As trap captures increase, *P. operculella* infestation in tobacco follows, starting on the lower leaves of the tobacco plant (Rivera, *in prep*). When scouting, mines are difficult to find without searching the whole plant. Mines can be easily mistaken for abiotic damage or chemical burn, especially early mines (Figure 1.3). Mines are more visible by the 3rd or 4th (last) instar, are blotch-shaped, occur near leaf veins, and are translucent with apparent frass (Figure 1.2). Older, abandoned mines retain the blotch shape but the once translucent area turns brown and easily flakes away. Frass is generally still present. To assess infestation level for treatment purposes, scouts are encouraged to observe 20 sequential
plants in 10 random places in the field, noting the number of mines per plant with and without larvae present. At this point, there is no clear economic threshold but 10 active mines per plant is the current recommended treatment threshold.

Management options

Tobacco fields with a known history of *P. operculella* infestation should be avoided in rotation when possible. Two insecticides are labeled for use in tobacco against large infestations, flubendiamide (Belt™, Bayer Crop Sciences) and chlorantraniliprole (Coragen™, DuPont). Fields with early season (pre-topping) infestations should be regularly scouted and treated if a second or third larval generation occurs. Late season infestations below the recommended treatment threshold in flue cured tobacco should be addressed with timely harvest when feasible. Moving infested leaf material out of the field and into barns will cease *P. operculella* feeding and kill larvae. In burley, infestation below the recommended threshold should be treated before harvest as *P. operculella* will continue feeding in an air cured setting.

Future research

The primary need for research is the development of a clear damage relationship and threshold for *P. operculella* in burley and flue-cured tobacco. Secondarily, further research into the landscape ecology of this species in tobacco fields in the southeastern United States is needed. Adult behavior and distribution within the field is key to understanding how infestation spreads. Multiple greenhouse studies have shown oviposition occurring on the soil
in tobacco (Varela and Bernays 1988; Van Vuuren et al. 1998), but plant architecture likely
diffs between greenhouse-grown and outdoor grown, as well as differences as a result of
cultural practices in commercial tobacco. Adult moths may be more attracted to plants as
they flower and oviposition location may change as plant architecture changes in flue-cured
harvest. Within the field, solanaceous, and potentially other, annual weeds are also
potentially attractive to adults for oviposition. Once tobacco is harvested, identifying and
locating plants in the field where adults and larvae feed could reduce the field population
with the potential to reduce this reservoir.
REFERENCES


Traynier, R.M. (1975) Field and laboratory experiments on the site of oviposition by the


Table I.1: Development Thresholds (°C) of *Phthorimaea operculella* in Egypt

<table>
<thead>
<tr>
<th>Development Threshold (°C)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>13.75(^1)</td>
</tr>
<tr>
<td>Larvae</td>
<td>15.4(^1)</td>
</tr>
<tr>
<td>Pupae</td>
<td>10.4(^2)</td>
</tr>
<tr>
<td>9.5(^2)</td>
<td>9.93(^3)</td>
</tr>
<tr>
<td>7.6(^3)</td>
<td>10.98(^2)</td>
</tr>
<tr>
<td>Daoud et al. 1999</td>
<td>10.98(^2)</td>
</tr>
<tr>
<td>7.6(^3)</td>
<td>10.5(^1)</td>
</tr>
<tr>
<td>7.79(^3)</td>
<td>10.98(^2)</td>
</tr>
</tbody>
</table>

\(^1,^2,^3\): numerical order from highest to lowest
Figure I.1 Female pupae (A) differs from male (B) pupae by having the small incision or “scar” on the 9\textsuperscript{th} abdominal segment closer to the tip of the pupae rather than the 8\textsuperscript{th} segment. In the adult stage, females (C) have a slim abdominal tip compared to the male (D) abdominal tip.
Figure I.2. *P. operculella* damage to a young tobacco plant early in the growing season. Photo via Sterling Southern, used with permission.
Figure I.3. *P. operculella* mines of various ages on tobacco leaf (A) Young mine from top-side of leaf, darker area is frass filled. (B) Young mine from underside of leaf, darker area is freass (C) Large late mine, with necrotic, darkened tissue.
Figure I.4. Pheromone trap placed near tobacco field.
CHAPTER I

Phenology of the Potato Tuberworm, *Phthorimaea opercuella* (Zeller), in the Tobacco Agroecosystem

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Phthorimaea operculella (Zeller), the potato tuberworm, is an infrequent, but potentially serious, pest of tobacco. The oligophagous larval stage mines the leaves of tobacco plants, and at high infestation levels, causes economic loss to growers by decreasing weight and quality of leaf material. The use of pheromone traps to track male P. operculella flight has been employed in the potato agroecosystem and is utilized in this study to track the phenology of P. operculella in tobacco. Traps were deployed over multiple locations in Virginia, North Carolina, South Carolina and Georgia. At each site North Carolina site, infield damage data was also collected and compared to the trap captures. Larval infestation in tobacco appeared 2 to 4 weeks following a peak in adult trap captures in North Carolina. Larvae were found first and populations were largest in the lower third of the plant. As the season progressed, larvae moved upward and were found in the middle and uppermost third of the plant. Using accumulated degree-days based on a lower threshold of 10°C, we determined 2 to 4 adult flights of P. operculella occur per tobacco growing season with an average generation time of 387 degree days. In North Carolina tobacco fields, 2 to 3 generations were observed based on larval infestation.
INTRODUCTION

Tobacco is an occasional host of *Phthorimaea operculella*, commonly known as, the potato tuberworm, the most important pest of potato worldwide (Graft 1917). Larvae of this species feed on oligophagously on solanaceous plants, including tobacco, on which it is called the tobacco splitworm. Adults are nocturnally active small brown moths (approximately 0.95cm long with a 1.30 cm wingspan) (Attia and Mattar 1939; Broodryk 1970, 1971; Traynier 1975, 1983). Under optimal conditions (30°C), *P. operculella* can complete 18 generations per year, requiring only 322.4 degree days (°C) per generation (Kabir 1994; Foot 1979). Each female moth is capable of laying 200 or more eggs in her lifetime, contributing to the potential severity of this pest (Moregan and Crumb 1914; Fenemore 1979).

The larvae of *P. operculella* bore into belowground potato tubers, but act as a leaf miner on tobacco. Larvae create blotch-shaped mines tobacco leaves, removing photosynthetic tissue and leaving only the upper and lower epidermis, resulting in a “window-pane” appearance. This damaged leaf tissue dies and leaves the leaf with a split appearance, reducing quality and therefore, causing economic loss. As in-field population increases, ten or more mines can develop on an individual tobacco leaf which can reduce the overall harvest weight, causing direct loss.

In recent years, tobacco growers in the southeastern United States have noted increasing damage by *P. operculella*. Infestations are sporadic from year to year and range from mild to severe. *P. operculella* has no larval diapause and, most likely, is surviving southeastern winters in the adult and egg stage (Foot 1979). Populations persist in potato
fields from year to year (Hanafi 1999), but growers who rent land may be less likely to know
the history of *P. operculella* in a field. Understanding the phenology of *P. operculella*
populations infesting tobacco will assist in predicting potentially severe infestations.

We used pheromone traps (Kennedy 1975) and accumulated degree-days (Foot 1979)
to observe the phenology of *P. operculella* in tobacco in 4 southeastern states. Adult male *P.
operculella* were captured throughout the winter, indicating adult survivorship. We observed
two to four *P. operculella* adult flights and 2 to 3 larval generations in southeastern tobacco
fields, with the potential for more generations increasing with warmer temperatures further
south.

**MATERIALS AND METHODS**

Five to seven tobacco fields in North Carolina, Virginia, South Carolina and Georgia were
monitored for *P. operculella* in 2008, 2009, and 2010 (Table 1). Of these locations, sufficient
*P. operculella* were present for multiple years at only 5 locations (Table 1). Four pheromone
traps (Pherocon® IC Trap and Pherocon® Cap, Trece Inc.) per site were used to monitor *P.
operculella* presence. Phermones attract male *P. operculella*, and the few non-target moths
captured in our traps were easily distinguishable. Trap bottoms were changed weekly and
pheromone lures changed every 4 weeks. Traps were suspended from wooden stakes 1 m
above the ground around field edges. This trap configuration was found to be the most
attractive in California potato fields (Kennedy 1975). Traps bottoms were collected every 7
days during the tobacco growing season. Male moths per trap were counted and divided by
the number of traps collected and amount of days the trap was in the field and expressed as moths captured per day.

Moths per trap per day were graphed for a subset of sites to determine the number of adult flights. Adult flights were identified by relating male moth trap capture peaks to degree day accumulation. In North Carolina, Chowan and Bertie County sites were chosen because both locations had very high populations in one or more years. Tift County, GA, Mecklenberg County, VA, and Fairfield County, SC were chosen from the many locations in the three other states because they had trap captures over all three years for comparison. Cumulative degree days using a lower threshold of 10°C (Foot 1979) were calculated for the duration of trapping for each site and year. Daily degree days were calculated by averaging the daily minimum and maximum temperatures and subtracting the lower threshold and were summed to calculate the cumulative degree days over time. Generation time in potato using a lower threshold of 10°C was 322.4 degree days (Foot 1979), and this value was used as a guide for determining when adult flights occurred in tobacco. Although we collected daily temperature and humidity data in the field using WatchDog Data Loggers (Model 150, Spectrum Technologies, Plainfield, IL), it was necessary to utilize weather data obtained from the North Carolina Climate Retrieval and Observation Network of the Southeast (NC CRONOS) database (State Climate Office of North Carolina 2011) for calculation due to gaps in collected data. Annual degree day accumulations were begun on 1 February for all sites, and moth captures for each year were graphed with a start date of 1 June for all sites.
At North Carolina sites, plant transects in the tobacco field were monitored in conjunction with pheromone traps. Plant transects near each trap were established within 1 month of transplant. Transects consisted of three rows of 100 plants in 3 to 4 locations near the pheromone traps (900 to 1200 plants per site), depending on field size. Each plant was observed for mines and mine location (middle, lower, or upper third of the plant). Each mine found was opened to determine if larvae were present or absent. Any live larvae found were destroyed to ensure they were not counted in subsequent surveys. Mine location data for all years from the Bertie and Chowan County sites were pooled, and an analysis of variance was conducted for each county via Proc Mixed (SAS v. 9.1.3 SAS Institute, Cary, NC) with month and mine location as fixed independent variables and week during each sample year as a repeated independent variable. Proportion data were arcsine transformed to meet assumptions of normality, and means were separated via LSD.

Larval *P. operculella* infestation in North Carolina was highly variable, and moths were captured at locations with little or no larval damage tobacco. However, in locations with in-field damage (Chowan and Bertie County sites), larval density was graphed along with adult *P. operculella* activity to confirm association of adult flights (moth captures) with larval feeding in tobacco.
RESULTS

Peaks in male moth captures with 322.4 or more accumulated degree-days between them were considered adult flights (Table 3).

North Carolina

In NC, two to four adult flights per year in tobacco were observed (Table 3), and 2008 was the only year with four flights in North Carolina (Table 3). In the sites with sufficient in-field pressure, two to three larval generations were observed. Increases in moths captured occurred one to two weeks before observed larval infestation began (Figure 1, Figure 2) and three to four weeks before larval infestation peaked. In the two NC sites with large larval infestations, the number of *P. operculella* mines was significantly higher in the lower third of the plant in the early part of the season and then moved up and through the plant in the later season (Table 2).

Virginia, South Carolina, and Georgia

*P. operculella* were caught over multiple years in all states. Including sites with very low trap captures was unavoidable with Virginia moth capture data, but otherwise, sites with three years of data and high captures were utilized. Moth captures in Georgia illustrated two to four flights per year and a rapid increase in captures toward the end of the tobacco growing season (Table 3). Flights two and three in Georgia and South Carolina appeared to overlap in 2008 (Table 3). Two to three flights occurred in South Carolina and one to three in Virginia. Virginia was the only state with less than two flights per tobacco growing season.
DISCUSSION

Temperature differences and local population between states likely contributes to plasticity in the number *P. operculaella* adult flights and larval generations in tobacco by state and year. Moth captures by pheromone traps and degree days are both commonly used to estimate the number of generations of insect species but can be confounded by many factors including pheromone attraction of distant insects and weather. Larval infestation data from in-field observation is the only way to confirm the insect presence in the crop or plant of interest, and observations from North Carolina support this distinction. Overall, populations in North Carolina, South Carolina and Georgia were very similar in number of adult flights per tobacco season. Most locations had three adult flights per year, but the number of moths captured and infestation rate vary. Virginia was the only state to have only one apparent flight in a season but also had relatively low trap captures. When male trap capture peaks were compared to accumulated degree days, in every instance, the total accumulated degree days were higher than 322.4, calculated in potato (Foot 1979). Currently, there is no calculated upper development threshold for *P. operculaella*. Our observations suggest that an upper threshold may be biologically meaningful in the southeastern United States and may improve predictions of degree day based phenology models were they to be employed for this pest. Additionally, larvae may be exposed to higher temperatures inside a sunlight-exposed mine than ambient air temperature.

Sites in Virginia had the lowest winter temperatures and the most mild summers, while Georgia had higher summer temperatures and the most mild winters. Tobacco is
transplanted later in the calendar year in Virginia and the earliest in Georgia. Early season *P. operculella* populations in Georgia and South Carolina appear to build from nothing to high numbers in the later part of the season (Figure 3), as is true with North Carolina trap captures (Figure 1, Figure 2). This slow start could be the result of low winter survival and movement into fields or in instances where trap captures stay very low, the attraction of surviving adults from nearby feeding sites by the pheromone. In 2008, Georgia and South Carolina’s second and third flights overlapped, meaning adults from both generations were most likely in the field together for a short period of time (Table 3). Moth trap captures persisted in Georgia and South Carolina late into the year in all years (Figure 3), which suggests adult activity and survival through the winter, potentially on alternate hosts.

*P. operculella* have no known diapause for any life stage. Warmer temperatures in Georgia and South Carolina may be more suited to the survivorship of alternate hosts in and around tobacco fields, and thus increased *P. operculella* survivorship throughout the year. *P. operculella* is a frequent pest in Georgia from year to year (J. Michael Moore, personal communication). Conversely, *P. operculella* is an infrequent pest in Virginia. Populations in Virginia tobacco fields were not large, and this may be linked to limitations of *P. operculella* survivorship. Survivorship through the winter is essential for successful infestation in the following tobacco season; tobacco plants are only in the field, from planting to harvest, for four to five months.

Although *P. operculella* larvae need living plant material to survive, eggs and adults may survive in sheltered areas. In areas with relatively more mild temperatures, like Georgia,
it is possible that *P. operculella* are surviving in all stages during the winter months. Tobacco is not as common or concentrated in Georgia, South Carolina or Virginia as in North Carolina. *P. operculella* populations outside of North Carolina may be less suited to exploiting tobacco which may explain why moth captures were not consistently high from year to year (Singer et al. 1988; Thompson 1994). Tobacco production is concentrated on mostly family farms in a small area in North Carolina. The concentrated nature of flue-cured tobacco production in North Carolina is illustrated by 5 counties in eastern NC, Wilson, Johnston, Edgecombe, Nash, and Wayne Counties, which produce the most of this type of tobacco in all of the United States (USDA, 2008). We monitored *P. operculella* in Nash County but found the largest infestations further east in two costal counties, Chowan and Bertie. Coastal areas are often more humid and thermally stable, and, coupled with sandy soil, these factors may promote better survivorship in this areas. Sandy soils drain water quickly and *P. operculella* egg survivorship is influenced negatively by moist soils (Van Vuuren et al. 1995).

The concentration of tobacco in North Carolina suggests that moths may not need to travel large distances, even with crop rotation, to find new tobacco fields from year to year. In Chowan County in 2009 and 2010, the tobacco field monitored was moved across a dirt path, far less than 240 m, the current known dispersal distance for adult *P. operculella* (Cameron, et al., 2009). In 2009 in Chowan County, the observed in-field damage reached almost 100%, with a mine on every plant (900 plants), but was much lower in 2010 (Figure 1b and 1c). The difference in infestation level may have been influenced by other factors such as plant health, irrigation, and weed or alternate host presence.
In Bertie County North Carolina in 2009, \textit{P. operculella} captures steadily increased through the growing season. The Bertie County field used in 2009 was different from the field used in 2010. The 2009 location shows small infestation and is a good example of what a normal \textit{P. operculella} infestation looks like from year to year, present but not in high numbers. Bertie County in 2010 illustrates an infestation quickly increasing to damaging levels. It appears in 2010 that two small larval infestation peaks occurred close together before the observed infestation reached 100%. Moth captures, the peaks are less obvious, but when calculating degree-days, they match up according to the infield infestation. The obscurity of the peaks is the result of adults from the two small larval peaks being active in the field at the same time, blurring the adult decrease that usually make the peaks more obvious. The resulting larvae from both these flights contribute to the very large late peak in larval infestation. Larval success during the 2010 could be attributed to increased survivorship of eggs in this field due to very hot weather that kept soils dry (Van vuuren, et al. 1995).

North Carolina sites appear similar to South Carolina and Georgia than to Virginia. In Virginia, \textit{P.operculella} seems to vary in success by year and likely will be more of a potential threat in years with mild winters and no snowfall. Winter survival likely occurs in GA, SC, and NC but local populations may have to move from distant fields, limiting their ability to rapidly increase in the early season. Once established in a tobacco field, infestations can increase quickly over short periods of time, often weeks. Pheromone traps may be useful to assess local populations during a growing season, but moth captures do not necessarily result in larval infestation. Due to the unpredictable nature of larval infestation, efforts should
be made to identify and quantify risk factors that can be combined with adult monitoring to
determine when a tobacco field is likely to experience significant damage from *P. operculella*. Our monitoring and climate data can then be employed to construct phenology models, which may be useful in predicting when damage will occur.
REFERENCES


(Dogrammaci and Tingey 2009).


Table 1.1. *Phthorimaea opercul ella* pheromone trapping locations by year, state and county*

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*Sites in **BOLD** included in analysis.*
Table 1.2 Proportion of mines observed in upper, middle and lower regions of the tobacco plant in Bertie and Chowan Counties, North Carolina.

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<sup>1</sup> Means within a month followed by the same letter are not significantly different (α = 0.05), F = 27.96<sub>6,69</sub>, p < 0.0001.

<sup>2</sup> Means within a month followed by the same letter are not significantly different (α = 0.05), F = 8.17<sub>4,27</sub>, p = 0.0002.
Table 1.3. Generation length for distinguishable flights. Flights without clear beginnings and ends were excluded.

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<th>2</th>
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<th>Maximum moths/trap/day</th>
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<td>329*</td>
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<td>Chowan</td>
<td>2010</td>
<td>485</td>
<td>436</td>
<td></td>
<td></td>
<td>461</td>
<td>29</td>
</tr>
</tbody>
</table>

**Grand Average** 387

* indicates overlap in adult flights.
Figure 1.1. Chowan County, NC 2008 (A), 2009 (B), 2010 (C) trap captures and proportion of plants infested over time. Arrows indicate the accumulated degree days for the following trap capture peak.
Figure 1.2 Bertie County, NC trap captures and proportion of plants infested from 2009 (A) and 2010 (B). Arrows indicate the accumulated degree days for the following trap capture peak.
Figure 1.3. Trap captures for Tift County, GA, Nottoway County, VA and Florence County, SC for 2008, 2009, and 2010
CHAPTER II

Performance of Potato Tuberworm, *Phthorimaea opercuella* (Zeller) Larvae on Tobacco by Location within Plant and Plant Age.

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ABSTRACT

The potato tuberworm, *Phthorimaea operculella*, an oligophagous herbivore of solanaceous plants, has recently become a concern for tobacco growers in the southeastern United States. Tobacco (*Nicotiana tabacum*) is an infrequent host of *P. operculella* and few studies are available on the biology and ecology of this pest in the tobacco agroecosystem. Feeding habit and oviposition preference differ between potato, its primary host, and tobacco. In potato, *P. operculella* oviposits on leaves and exposed tubers, larvae bore into tubers when available, and occasionally mine leaves. On tobacco, oviposition occurs in the soil, and larvae are largely restricted to leaf mining. Foraging in potato to exploit both tubers and leaves differs from the primarily vertical foraging in tobacco. In tobacco, more larvae establish in the lower leaves under field conditions. In this study, we restricted larvae at different vertical levels in the tobacco plant at discrete plant ages to determine if establishment in the lower leaves offered a performance advantage or if lower leaves just provide quicker access to leaf material. We found larvae perform better on the higher, younger tobacco leaves, resulting in a performance trade off between larval host selection.
INTRODUCTION

*Phthorimaea operculella* (Lepidoptera: Gelechiidae) populations in tobacco production areas are increasingly found mining the leaves of tobacco. The larval stage is one of the most important pests of potato in the world, on which it is commonly known as the potato tuberworm. *P. operculella* has a worldwide distribution, but is endemic to western South America, where it is thought to have formed its association with potato plants (Graft 1917). Tobacco, like potato, is commercially cultivated but is native to North America, where *P. operculella* is a non-native pest species. The literature regarding *P.* operculella largely describes its biology and ecology around its association with potato and provides very little information on interactions with tobacco.

Compared to other solanaceous plants, cultivated tobacco plants have unique architectural traits. Tobacco plants are tall with large, vulnerable leaves protected by visually absent plant-derived protection (Mattson, 1980). The “size per se” hypothesis suggests large plants are more likely to be found and colonized by insects, but in the tobacco agroecosystem, plant apparentcy is limited by the short annual tobacco season (Connor and McCoy 1979). *P. operculla* is an infrequent pest of tobacco but has had a more significant presence in the tobacco agroecosystem in recent years (Rivera, in prep). Larvae mine the leaves of tobacco, creating a “blotch mine”, leaving only the upper and lower epidermis. This results in a windowpane appearance of the leaf. On tobacco leaves, this area decomposes, resulting in leaf splitting. Although *P. operculella* larvae also mine leaves on potato plants, economic damage is caused by their proclivity for boring into tubers both in the field and in
storage. This injury results in an unmarketable product. In tobacco, the marketability of mined leaves varies with level of infestation; leaves with many mines may result in economic loss for growers based on reductions in weight and quality.

_P. operculella_ has high reproductive potential, producing up to 18 generations per year under optimal conditions (Kabir 1994). The ability to adapt to a wide range of temperatures gives _P. operculella_ a competitive advantage compared to other species (Foot 1979, Dangles et al. 2008). However, _P. operculella_ does not diapause, and larvae needs host material after cultivated hosts are moved from the field (Broodryk 1971).

Two types of tobacco, burley and flue-cured, dominate the market and are grown in the highest volumes by Kentucky and North Carolina, respectively (NASS, 2008, NASS, 2010). Burley and flue-cured tobacco differ most notably in harvest and curing procedures. Growers are responsible for growth and curing of tobacco before it is graded, sold, and processed. Flue-cured tobacco is harvested by removing the leaves from the stalk, grouping by stalk position, and cured by exposure to heat. In burley tobacco, the entire burley plant is removed and hung in a barn to air cure with no heat source. _P. operculella_ was first acknowledged by southeastern tobacco growers as a minor, early-season pest, occurring shortly after transplant and in association with potato production. Recently, significant late season splitworm infestations have appeared, and in some cases have reached levels causing economic loss. The chemical properties of tobacco leaves vary based on position within plant and by plant age. Quality and price are based on stalk position. Alkaloid, of which nicotine is the most common, content varies in harvested and cured leaf material. The uppermost
leaves are the best defended on the tobacco plant, have the highest alkaloid content, and command the highest price.

The cultural practice referred to as “topping” removes the floral buds and first few leaves from the top of the plant. This releases apical dominance and promotes the increase of alkaloid concentrations. Growers fertilize tobacco crops after transplant to promote growth but do not re-apply fertilizer in the later part of the season, and this depletion of available soil nitrogen further increases alkaloid production (Goenaga et al. 1989). After topping but before harvest, the plant depletes the soil-nitrogen to the point of nitrogen starvation. Nitrogen starvation induces premature senescence, which reduces plant vitality and decreases the quality of the leaf material for tobacco feeding herbivores. Fertilizer regimes differ between burley and flue-cured plants, with burley receiving more nitrogen than flue-cured but in both types (NCSU, 2010). Tobacco-feeding herbivores may be exposed to different alkaloid levels, depending on their seasonal biology and within plant feeding niche.

Female *P. operculella* moths oviposit in the soil near tobacco plants rather than on the leaves (Varela and Bernays 1988, Van Vuuren et al. 1998). Tobacco leaves may not be suitable for oviposition due to glandular trichomes and leaf surface chemistry (Jackson et al. 1984; Van Vuuren et al. 1995). Females have mechano- and chemo-receptors on their ovipositor, oviposit preferentially on more hairy surfaces and surfaces with egg-fitting depressions, and are more fecund in response to potato tuber extract (Fenemore 1979). Laying eggs away from the food source, tobacco leaves, exposes eggs to soil moisture which
can be a source of mortality (Van Vuuren 1998) and potentially, further exposure to predation while larvae are en route to feeding site.

Oviposition on the soil in tobacco divorces adult host-selection site from larval feeding site selection. Larvae not only need to navigate from the oviposition site to the plant but also up the plant stem to a suitable feeding site on the foliage. On the plant, larvae must cope with both physical and chemical defenses. Each leaf emerges with a set number of trichomes, with the youngest, most alkaloid-abundant leaves having the greatest trichome density on the plant (Dawson 1945). We hypothesized that *P. operculella* would feed less and perform worse on higher stalk position leaves and younger tobacco plants due to higher trichome density and alkaloid content in the uppermost leaves and perform better on lower stalk position leaves where they are commonly found under field conditions. We found *P. operculella* experienced performance differences by plant age and stalk position. The youngest plants and leaves yielded the highest pupal weight and fastest larval development rate, which suggests larvae do not gain a performance advantage by feeding on lower leaves.

**MATERIALS AND METHODS**

To test the effect of stalk position and plant age on *P. operculella* performance, we restricted 10 neonates to upper, middle, and lower leaves 2 times in the 2009 season and 3 times during 2010. A splitworm colony originating from insects collected from tobacco plants but maintained on potato for > 62 generations at a constant temperature of 30° C (+/- 2) and humidity of 60% RH (+/- 2) was used for this research.
This study was conducted at North Carolina Department of Agriculture & Consumer Services and North Carolina State University research stations, in Clayton, NC and Oxford, NC, respectively. Tobacco was managed according to commercial standards found in the Flue-cured and Burley Tobacco Guides (NCSU 2009-10). Greenhouse grown tobacco plants (var. K326) were transplanted with a 61 cm plant spacing and a 1.2 m row spacing in the third week of April both years. Plants were topped in both years during the first week of July. Each row had 30 plants in 2009 and 50 plants in 2010.

In 2009, 10 replicates of each of the three stalk positions were placed at two times, 25 June, before flowering, and 25 July, after topping. On the second date, 25 July, an uncaged control was also included to assess cage effect on insect performance. With this treatment, 10 larvae were placed on leaves as in caged treatments, but a cage was not placed on the infested leaf until late in larvae development when the cage was needed to collect larvae exiting the mine. In 2010, 15 replicates of each stalk position were placed three times, 29 June, before flowering, 29 July, after topping, and at harvestable maturity in the lower leaves, 24 August. On 29 July 2010, 15 replicates per stalk position were also placed on Burley (var. NC 7) tobacco.

We released 10 neonate larvae on one leaf per plant in the upper, middle or lower third of the plant. Three adjacent rows were chosen for each placement date, plants were selected within these rows based on leaf quality (fully intact with little to no damage by other insects), and replicates of each stalk position were evenly distributed over the three rows. At least one non-infested plant was left between caged plants within rows. Larvae were
transferred to the underside of the leaf using a paintbrush and caged with drawstring bags made of organdy mesh to restrict larvae to the chosen leaf and exclude predators and parasitoids. Larval establishment, as indicated by mines per leaf, was recorded and caged leaves were checked daily for larvae that had exited their mines. Pre-pupae were obvious in the cages because they attached their silk case to the cage fabric, usually along the seams. Pre-pupae were removed, carefully put into microcenterfuge tubes, transported to the lab, and placed in a climate-controlled environment (30° C, 60% RH). When larvae pupated, usually on the same day they were collected, sex and weight of the pupae were recorded and development was tracked until adult emergence. Life history parameters were recorded for each larva brought into the lab. We recorded larval development time, pupal duration, pupal weight, sex, and days until adult emergence. Degree days (°C) accumulated during the time larvae were in the field were calculated using weather data from North Carolina Climate Retrieval and Observation Network of the Southeast (NC CRONOS) database (State Climate Office of North Carolina 2011).

Analyses were performed using SAS statistical software (SAS Institute 9.1.3, Cary, North Carolina, USA). A mixed linear model (Littell et al. 2006) was constructed with stalk position, caging date, year, and all interactions as fixed, independent variables. Replications were included as random effects, with sex as a covariate.
RESULTS

Pupal weight and larval development time were significantly different between both years by plant age (placement date: early, middle, late) and stalk position. Pupal duration was not significantly different by plant age or leaf position.

2009 Field Season

*P. operculella* performance, measured indirectly as pupal weight and larval development time, decreased on older plants that had flowered and been topped. Pupal weight differed significantly by plant age and stalk position. Pupae of larvae feeding on pre-flowering, younger plants weighed more than the pupae of larvae placed on plants after flowering and topping (F = 24.77, p ≤ 0.0001, df=2, 133) (Figure 3.1). Pupal weight also increased when larvae fed on middle and upper leaves as compared to lower leaves when averaged over both placement dates. Larval development time decreased when splitworms fed on younger plants (F= 5.24 p=0.0335, df= 1, 19) (Figure 3.2). Means calculated for pupal weight and larval development time of uncaged larvae did not differ significantly from the caged larvae (Figure 3.1, Figure 3.2).

2010 Field Season

Pupal weight differed significantly by plant age and was higher in the early than in the late season placement date (Figure 3.7) (F=11.13, p=≤ 0.0001, df =3, 72.8). Average pupal weight varied significantly between stalk positions in the burley and late season placements in flue-cured. In both the mid-season placement date burley and late season flue-cured, upper
stalk position pupal weights were significantly higher than on the lower stalk position (Figure 3.4, 3.8) \((F = 3.65, p = 0.0421, \text{df} = 2, 22.6; F = 4.12, p = 0.0411, \text{df} = 2, 13)\). When flue-cured and burley data from all placement dates were averaged by stalk position, larvae developing on upper and middle stalk position leaves were significantly heavier than those developing on lower stalk position leaves (Figure 3.3) \((F = 6.81, p = 0.0019, \text{df} = 2, 71.9)\). Larvae developed quicker on early, pre-flowering plants than on late season mature plants (Figure 3.7) \((F = 42.06, \text{df} = 3, 84, p = \leq 0.0001)\) but did not differ by stalk position within any of the placement dates.

Larvae feeding on burley tobacco after topping had a higher pupal weight than those feeding on flue-cured tobacco but there were no significant differences in larval development time. Within burley plants, larvae developing on upper stalk leaves were heavier (Figure 3.4) \((F = 3.65, p = 0.0421, \text{df} = 2, 22.6)\). Larvae developing on burley did not develop any quicker than those on flue-cured (Figure 3.5) nor did larvae differ in development time when placed at different stalk positions within a burley plant \((F = 1.04, p = 0.3728, \text{df} = 2, 19)\).

**Combined Data Sets**

Pupal weight differed by stalk position when data were combined over both years (Figure 3.6) \((F = 3.28, p = 0.0467, \text{df} = 2, 46)\) with the middle stalk position producing larval weights significantly higher than the lower stalk position but not higher than the upper stalk position. Larval development was significantly quicker in the early placement dates (pre-flowering) over both years (Figure 3.7) \((p = 0.0021, \text{df} = 1, f = 52.7)\). Fewer degree-days were accumulated
during the early season when significantly less time was take for larvae to cycle through to pupation (Figure 3.5).

DISCUSSION

Although *P. operculella* larvae are most common in the lower region of tobacco plants in the field, larvae developed faster and resulted in larger pupae when feeding on younger leaf tissue, contrary to our hypothesis. *P. operculella*’s reduced performance on lower stalk position leaves and older plants could be the result of negative nutritional effects due to decreasing nitrogen availability as the growing season progresses (Mattson, 1980). Insects, like plants, require nitrogen but use it less efficiently than plants due to losses in frass. Partitioning of nitrogen in tobacco is correlated with growth rate and is highest in the uppermost, new leaves (Goenaga et al. 1989). In the tobacco agroecosystem, fertilizer is applied in the early season to increase soil nitrogen content for rapid, healthy growth. While within plant distribution of nitrogen will be highest in the uppermost leaves throughout the season, the uppermost leaves where nitrogen is accumulated at the highest rate are removed with the reproductive structures mid-season (Raper and McCants 1966; Mattson 1980). Declining host quality could be associated with our observation of decreasing *P. operculella* performance in the later part of the season. Plant age treatments as they were used in this study are confounded by seasonal change. However, differences in degree-days accumulation between caging periods (Figure 3.5) suggest that factors other than temperature influenced development rate.
Larval feeding in the lower leaves may be due to oviposition on the soil surface. Plant traits, like nicotine content and trichomes, may hinder adult oviposition on tobacco leaves which would give larvae direct access to higher quality leaf material (Jackson et al. 1984; Van Vuuren 1995). The foraging habit of neonates in potato may translate to tobacco. While the uppermost leaf material may be the best food source in tobacco, lower leaf material is suitable and provides a source of food and protection closer to the oviposition sites on the soil surface, than the upper leaves.

Larvae have been observed on upper stalk leaves when populations are large (Moregan & Crumb 1914). Observing mines only in the lower leaves may be an indicator of a small population. The cues neonates use to forage farther into the plant as well as supplementation of the in-field populations from surrounding areas are unknown. The total mine capacity of tobacco leaves is unknown but lower leaves should be able to support more mines than upper leaves.

Although *P. operculella* has been recorded to feed in tobacco as early as 1912 (Moregan and Crumb), they were not considered a potentially serious annual pest in the southeastern United States until recently. This has resulted in labeling of insecticides to control *P. operculella* in tobacco. Evidence from this study suggests, *P. operculella* are opportunistic feeders on tobacco and do not necessarily seek optimal plant parts where larval development and pupal weight would benefit. Removing infested lower leaves in a timely manner may reduce or eliminate larval infestations restricted to lower stalk leaves and thus, reduce in-field population. The heat of the curing barns used in flue-cured tobacco certainly
kill larvae however, in burley tobacco, splitworm infestations pose more of a threat. Burley is air-cured and in open, ambient-temperature barns *P. operculella* can continue feeding and complete development on the harvested plants. Leaf material will eventually become unsuitable in the barn, but depending on the level of infestation and age of larvae at harvest, this situation could become problematic if larvae are young and the plants are heavily infested. The larval foraging abilities and ability to survive low temperature allow survivorship through the winter. Consequently, sanitary practices, such as stalk disposal, in the field post-harvest and around curing barns in both flue and burley tobacco varieties should be encouraged.
REFERENCES


Figure 2.1. 2009: *P. operculella* Pupal weights by placement date and caging treatment on flue-cured tobacco. (F= 24.77, p ≤ 0.0001, df= 2, 133)
Figure 2.2. 2009: *P. Operculella* Larval development by placement date in flue-cured tobacco. (U= Upper, M= Middle, L=Lower) (F= 5.47 p=0.0078, df= 2, 41.8).
Figure 2.3. 2010: *P. operculella* Pupal weights by stalk position including both tobacco types. (F= 6.81, p=0.0019, df= 2, 71.9)
Figure 2.4. 2010: *P. operclella* Pupal weights by stalk position on burley tobacco plants. 
(F= 3.65, p=0.0421, df= 2, 22)
Figure 2.5. 2010: Degree-days accumulated between *P. opercuella* larval placement date and larval emergence from the mine for each placement date and stalk position within (L = lower, M = middle, and U = upper). Degree-days calculated for duration of time in field using weather data from NC CRONOS.
Figure 2.6. 2009 and 2010: *P. operculella* Pupal weights by stalk position including both tobacco types. (F=3.28, p =0.0467, df=2, 46).
Figure 2.7. 2009 and 2010: *P. operuclella* larval development by placement date including both tobacco types (F=10.46, p= 0.0021, df=1, 52.7).
Figure 2.8: 2010: *P. operculella* larval development by placement time and tobacco type (F= 42.06, df= 3, 84, p= ≤ 0.0001).
CHAPTER III

Reduced Establishment of Neonate Potato Tuberworm, *Phthorimaea operculla* (Zeller) as Distance from Oviposition Site is increased in *Nicotiana tabacum* Plants

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ABSTRACT

Potato tuberworm (*Phthorimaea operculella*) larvae feed on solanaceous plants. Potato (*Solanum tuberosum*) is the most common host of *P. operculella*, and larvae mine leaves and bore into tubers. In tobacco, larvae primarily act as leaf-miners. Multiple studies have shown adult female moths lay their eggs predominately in the soil in tobacco, which means larvae must move to find acceptable host tissue. The space between the soil surface and leaf material in tobacco is altered over time by leaf removal during harvest of flue-cured tobacco sequentially from the bottom of the stalk. We hypothesized that the increased distances that *P. operculella* must travel to find suitable leaf tissue in tobacco following harvest of lower leaves would decrease larval establishment. Results indicate this is true but other factors impact larval foraging and survivorship in addition to the distance neonates must travel to find suitable host material.
INTRODUCTION

The potato tuberworm, *Phthorimaea operculella* (Zeller), an insect known worldwide for damage to potatoes, is a recent concern to tobacco growers in the southeastern United States. *Phthorimaea operculella* larvae are oligophagous on both cultivated and wild solanaceous plants (Broodryk, 1971, Fenemore 1988) and have been known to infest tobacco, where they are commonly referred to as the tobacco splitworm, in the United States since at least 1912 (Moregan and Crumb 1914). Despite early accounts of feeding in tobacco, *P. operculella* remained an infrequent, early season pest until 2007, when severe damage was reported late season in North Carolina, causing economic loss to growers. Previous research on the biology and ecology of *P. operculella* focused primarily on the potato agroecosystem (Rhondon, 2010). Adult moths are nocturnal, both copulating and ovipositing at dusk and through the night (Attia and Mattar 1939; Broodryk 1970, 1971; Traynier 1975, 1983). Under optimal conditions, *P. operculella* can complete 18 generations per year and if quality food is available, females will lay higher numbers of eggs, increasing population size dramatically until full utilization of the food source (Trehan and Bagal 1944; Labeyrie 1957; Traynier 1983; Kabir 1994). *P. operculella* phenology in tobacco has shifted from early season infestations locally associated with potato production to late season infestations in areas where potatoes are not present. This shift in seasonal biology has resulted in greater pest significance for tobacco. The drivers of this shift are currently unknown.

The tobacco agroecosystem is unique in its management and focus on plant chemistry and architecture. Two types of tobacco, burley and flue-cured, dominate the market and land
area and are planted in Kentucky and North Carolina, respectively. While in the field, tobacco is managed as a row crop until plants reach reproductive development. When flower buds appear, they are removed in a practice called “topping,” which impacts alkaloid concentration in the leaves (NCSU, 2010). Harvest of flue-cured tobacco, called priming, involves removal of leaves from the bottom of the plant upward, as they mature over the final weeks of the season. Harvested leaves are cured on the farm. In flue-cured tobacco, this involves placing leaves in heated barns until the moisture is removed and the leaf chemistry modified. Leaves are organized by stalk position because cured tobacco is graded in part on the basis of stalk position. Concentrations of plant defensive chemicals, including nicotine, are highest in the uppermost leaves on the plant and lowest in the lower leaves; upper stalk tobacco is thus typically more valuable than lower stalk leaves. Flue-cured harvest alters plant architecture over a large geographic area, changing the host tissue available to *P. operculella*. Harvest timing is based on leaf maturity and can be spread over 4 to 6 weeks, beginning as early as July and ending in September through October.

*P. operculella* larvae exploit a range of host plants and plant parts, sometimes feeding on multiple parts of the same plant, as on potato. Ovipositing moths select the host plant, but larval choice determines the plant part utilized. Movement of neonates is an important aspect of infestation in the commercial tobacco plants. A single female moth can lay 200 or more eggs, and in tobacco, they have been shown to oviposit almost exclusively in the soil (Trivedi and Rajagopal 1992; Varela and Bernays 1988; Van Vuuren et al. 1998). Neonates have previously been shown to travel only 8 cm up the tobacco stem on young, preflowering plants 30-60cm in height, establishing only in the lower leaves (Varela and Bernays 1988;
Rivera, in prep). However, in heavy field infestations, mines can be found at the top of plants over 1m tall (Moregan and Crumb 1914). Harvest in flue-cured tobacco sequentially increases the distance larvae must travel to reach suitable leaf material, increasing their susceptibility to predation and desiccation as the leafless open area is more subject to temperature changes (Varela and Bernays 1988; Van Vuuren et al. 1998). We hypothesized that alteration of the plant’s architecture, increasing the distance from oviposition location to leaf material, would decrease *P. operculella* establishment. The ability of larvae to travel from oviposition site to a suitable feeding site on the tobacco plant guides our understanding of establishment in the plant and ability to move to surrounding hosts. The objective of this study was to see if increasing the distance to the first leaf material limited establishment.

**MATERIALS AND METHODS**

Research plots were established at the Oxford Tobacco Research Station in Oxford, NC (2009) and at the Cunningham Research Station in Kinston, NC (2010). Greenhouse grown tobacco plants (var. K 326) were transplanted on April 30, 2009 (Oxford) and April 14, 2010 (Cunningham). Plants were treated with 17.76 ml imidacloprid per 1000 plants (Admire Pro, Bayer CropScience) in the greenhouse 2 to 5 days before transplant to control non lepidopteran leaf feeding insects. Unless otherwise noted, no foliar insecticides were applied to research plots. Leaves were manually removed from the bottom to the top of the plant after topping, in the latter part of the season. Leaf removal treatments were: Treatment 0: no leaves removed, Treatment 1: approximately 6 leaves removed (~30 cm from the soil surface), Treatment 2: approximately 12 leaves removed (~48 cm from the soil surface),
Treatment 3: 3 layers ~20 leaves removed (~58 cm from the soil surface). Treatments were arranged in microplots of three plants, isolated by removal of the adjacent plant on both sides and space between planted rows. Treatments were randomized within the field with 12 microplots per row. Treatments were replicated 30 times (Oxford 2009) or 25 times (Cunningham 2010). Larvae were placed on plants at Oxford in 2009 on August 28, 2009 and at Kinston, on August 17, 2010. In 2010. Plants were treated with *Bacillus thuringiensis* (*Bt*) (DiPel DF, Valent U.S.A.) to treat a hornworm infestation on August 12, 2010.

Larvae, obtained from a colony established from insects originally collected from tobacco and maintained for over 100 generations on potato, were placed at the base of the middle plant in each microplot using a small paintbrush. A total of 1,200 larvae were used in 2009 and 1,000 in 2010. Larvae were allowed to disperse freely. Establishment success, as indicated by an active larval mine, was assessed 3, 7, and 10 days after release. Distance from the larval release point to each larval mine was measured. The space between plants within the microplot was also measured, if the mine was on the first or third plant of the microplot rather than on the plant where the larvae were released,. These distances were used to estimate linear distance traveled by each individual larva. Distance measurements were taken on day 10 to ensure that only mines containing live larvae were assessed.

*Statistical Analysis*

Analyses were performed using the statistical software package SAS® 9.1.3 (SAS Institute, Cary, North Carolina, USA). A mixed linear model (Littell et al. 2006) was fitted for distance from larval release point to leaf mines at day 10 after release, with treatment, plant
position and their interaction as fixed effects. Replicates within each treatment, and replicates by plant position within each treatment were considered as random effects, to account for multiple measures for each mine on a plant. Residual variability was modeled separately for each treatment to account for residual variance heterogeneity. Simple effects were analyzed when interaction effects were significant. A pairwise t-test with Tukey’s adjustment was used for least squares means separation when relevant effects were significant. Data for each year correspond to different locations. A combined analysis for both years (or locations, since location was different each year) was run with location, treatment, plant position and their interactions as fixed effects. Replicates within each year and replicates by plant position within each year and treatment were considered as random effects. Numbers of mines were analyzed using the GLIMMIX (SAS Institute Inc. 2006) procedure in SAS®. A Poisson regression with the same variables as used for previous analyses was conducted. A t-test was used to test null hypothesis of no pairwise differences between treatments least squares means. The Tukey range test was used to control Type I Error. Combined analysis of the number of mines for both years included location, treatment, plant position and their interactions as fixed effects. Replicates within each year and replicates by plant-number within each year and treatment were considered random effects.

**RESULTS**

In 2009, mines per plant differed by treatment (p= 0.0038, df= 3/112.9, F=4.73) (Figure 4.1). Over the four treatments, the largest number of mines was present in Treatment 0, while each successive leaf removal treatment had fewer mines (Table 4.1). Larvae traveled
differing distances to reach leaf material in each treatment, traveling the farthest to leaf material in Treatment 3 (p=0.0038, df=3/112.9, F=4.73) (Figure 4.1). In the 2010 season (Figure 4.2), mines per plant also differed significantly by treatment (p=0.0067, df=3/94, F=4.33) as did linear distance traveled, which increased by treatment as in 2009 season (p=0.0038, df=3/112.9, F=4.73). Mines per plant within microplot did not differ significantly (Table 4.3).

DISCUSSION

Plant architecture manipulation impacts, but does not prevent, *P. operculella* establishment. In both years, lower number of mines formed in Treatment 3 than Treatment 0 (Figure 1, Figure 2). Though not significantly different, average distance traveled is numerically less in 2010 in every treatment when compared to 2009 (Table 1, Table 2). Differences in establishment and travel between years may be due to plant quality. In 2010, the plants used in the study were treated for a hornworm infestation on August 12, 2010 before manual infestation of *P. operculella* on August 17, 2010. Tobacco hornworm (*Manduca sexta* L.) feeding can cause the tobacco plant to respond defensively with changes in plant chemistry (Jongsma et al. 1994), possibly decreasing suitability for *P. operculella* establishment. Neonate larval establishment may depend also on microclimate and temperature. Overall, the average daily temperature for the duration of these experiments, were 25.8°C in 2010 in Kinston, NC and 21.7°C in Oxford, NC in 2009. High temperatures may increase the risk of larval desiccation.
Neonate *P. operculella* were released at the base of the center plant in each microplot but establishment did not differ significantly between the three plants (Table 3). The shortest distance to leaf material from the point of release was the lowest leaves on the release plant; however, larvae traveled into all three plants in microplots. The number of observed mines on all three plants within a plot never exceeded 10 and thus, there is no direct evidence to suggest movement between microplots. If there was movement between microplots, we have no way of verifying this movement. However, a previous study by Varela and Bernays (1988) found that only 50% of neonate *P. operculella* placed as eggs in the soil 0.5 cm away from the plant stem found tobacco in an indoor setting, which in this case, suggests movement between microplots was unlikely. Unless females are laying eggs on leaves in commercial tobacco fields, establishment in the no leaf removal treatment during 2009 (Table 4.1) suggests more than 50% success is possible.

Larval foraging behavior may have evolved concurrently with both boring and leaf mining in potato. Female moths lay eggs singly or in clutches of up to 15 on both the soil and leaves of potato; however, larval performance is enhanced when feeding on tubers (Fenemore 1980, Rhondon, et al. 2008). When tubers are available via cracks in the soil, female moths will deposit eggs in or around the eye buds of the tuber, which are limited in number per tuber (Fletcher 1914, Graft 1917, Al-Ali et al. 1975, Rhondon et al. 2007). Otherwise, larvae access tubers through soil cracks and enter the tuber through the eye buds or areas of physical damage; the periderm, or cork-like outer layer of the potato, prevents larvae from establishing otherwise (Dogrammaci and Tingey 2009). Therefore, while there may be advantages to boring into tubers, larvae from soil deposited eggs may have to seek
foliage if there is no available access to tubers. This seeking behavior may be a survival
tactic used by neonates in alternate plant hosts such as tobacco.

Larvae in the 2009 study traveled shorter distances than larvae in 2010 and
established in higher numbers (Table 4.1, Table 4.2). In treatment 0, where no leaves were
removed, larvae still traveled long distances from the release point to their establishment site
on the plant. The intact plant may create better habitat for extended foraging and encourage
further movement. However, larval performance differs based on where larvae establish
within the tobacco plant (Rivera, Chapter II). This study shows larvae are capable of
traveling the distances necessary to reach higher leaf material where performance gains could
be attained, but concealment within a mine seems to be higher priority. Concealment within a
mine produces lower mortality rate versus the rate for exposed feeders (Zalucki, 2002), and
larvae are commonly observed feeding in the lower portion of the plant under field
conditions (Rivera, Chapter I). Larval establishment was double or nearly double when
comparing treatment 0 to treatment 1, where one layer of leaves was removed from the base
of the plant (Table 4.1).

The ability of neonates to travel relatively large distances within or from plant to
plant as has implications for coevolution (Thompson 1994, 1999). Generally speaking, adult
oviposition mediates the selection of plants by larvae, especially in non-generalist insects.
The ability of this oligophagous insect to move large distances may increase access to
alternate host plants or plant parts and the potential to locate enemy free space. In agricultural
settings, there is an intentional monoculture of plants and often, weed species that are both
common and annually available like the crop, are also solaceous. In tobacco, soil oviposition is sub-optimal for *P. operculella*, who feeds in the leaves. If females are ovipositing solely in the soil in the tobacco agroecosystem, as has been shown in an indoor setting, the prediction of 50% of larvae not finding tobacco (Varela and Bernays 1988) may be untrue. However, extensive foraging abilities of *P. operculella* larvae suggest foraging for feeding sites can occur in neighboring tobacco plants away the oviposition site or other plant species nearby (Karban 1997; Rand 1999). This leaves selection of feeding location primarily up to offspring rather than parent.

Larval movement has implications for the dynamic relationship of this organism to tobacco plants in commercial production system. Late season infestations in flue-cured tobacco could be managed by moving harvestable tobacco from the base of the plant out of the field as soon as possible as this will kill larvae and reduce population size in the field. However, harvest can only slow further infestation if the distance between the ground and leaf material is more than 50.8-63.5 cm which is equated to the removal of 12-15 leaves in this study (Table 4.2).
REFERENCES


(Dogrammaci and Tingey 2009).


Labeyrie, V. (1957) Influence de l'alimentation sur la ponte de la teigne de la pomme de terre


Table 3.1: 2009 and 2010: Total *P. operculella* mines per plant leaf removal treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2009(^1) n = 300 larvae</th>
<th>2010(^1) n = 250 larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>217</td>
<td>74</td>
</tr>
<tr>
<td>1</td>
<td>150</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>81</td>
<td>8</td>
</tr>
</tbody>
</table>

\(^1\)Numbers do not differ significantly.
Figure 3.1. 2009: Linear distance (cm) traveled up the tobacco stalk by *P. operculella* larvae and number of mines by treatment.
Figure 3.2. 2010: Linear distance (cm) traveled up the tobacco stalk by *P. operculella* larvae and number of mines by treatment.
Table 3.2: 2009 and 2010: *P. operculella* mines and distance traveled by larvae to feeding location from release point

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Distance(^2) (cm)</th>
<th>Mines(^3)</th>
<th>Distance(^4) (cm)</th>
<th>Mines(^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>44.24 a</td>
<td>1.43 a</td>
<td>50.43 a</td>
<td>1.15 a</td>
</tr>
<tr>
<td>1</td>
<td>54.96 b</td>
<td>1.09 ab</td>
<td>56.54 b</td>
<td>0.9 ab</td>
</tr>
<tr>
<td>2</td>
<td>55.75 c</td>
<td>1.08 b</td>
<td>59.79 c</td>
<td>0.61 bc</td>
</tr>
<tr>
<td>3</td>
<td>70.38 d</td>
<td>0.47 b</td>
<td>74.96 d</td>
<td>0.19 c</td>
</tr>
</tbody>
</table>

\(^1\)Means followed by the same letter do not differ significantly at \( p = 0.05 \) (Tukey-Kramer).

\(^2\)(\( p \leq 0.0001, \text{df}=3, f=22.31 \))

\(^3\)(\( p=0.0038, \text{df}=3/112.9, f=4.73 \))

\(^4\)(\( p\leq0.0001, \text{df}=3, f=19.57 \))

\(^5\)(\( p=0.0067, \text{df}=3,94, f=4.33 \))
Table 3.3. 2009 and 2010: *P. operculla* mines per plant by microplot plant position

<table>
<thead>
<tr>
<th>Plant position</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>0.878</td>
<td>0.5917</td>
</tr>
<tr>
<td>Center</td>
<td>1.1542</td>
<td>0.717</td>
</tr>
<tr>
<td>Right</td>
<td>1.0171</td>
<td>0.8279</td>
</tr>
</tbody>
</table>