OCCURRENCE AND BIOLOGY OF PESTS ASSOCIATED WITH *BRASSICA CARINATA* AND DEFOLIATION IMPACT

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2019

To my wonderful husband

ACKNOWLEDGMENTS

I thank the entomology lab at the West Florida Research and Education Center in Jay, Florida for their support during my thesis work. I would have been incredibly lost without the help of Latisa, Marcelo, and Chris.

I would like to thank Dr. Mulvaney's team. They aided us greatly in the planning, planting, and harvest stage, of *B. carinata* with resources and knowledge.

Finally, I want to thank Dr. Silvana V. Paula-Moraes for her support, expertise, and guidance throughout the process of graduate school.

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August 2019

Chair: Silvana V. Paula-Moraes
Major: Entomology and Nematology

Brassica carinata has the potential to become an economically important biofuel crop in the southeastern United States. This study documented the pest occurrence, pest canopy position, impact of defoliation on number pods/plant, number of seeds/pods, thousand seed weight, and some biological parameters of pests feeding on *B. carinata*. The study was performed at the WFREC, Jay, FL, during the 2017/2018 and 2018/2019 winter/spring crop seasons. Pest species occurring in *B. carinata* were documented by plant inspection within 16 genotypes of *B. carinata*. In each plot, one *B. carinata* plant was divided in three canopy zones and inspected for the presence of pests. The defoliation impact on *B. carinata* was evaluated in an artificial defoliation study. The plants were hand harvested and the average number of pods/plants, seeds/pod, and thousand seed weight were determined. *Trichoplusia ni* and *P. xylostella* biology was documented in a feeding study on four genotypes of *B. carinata*. The study was conducted in growth chambers at 19.5 ± 2 ºC, relative humidity of 70 ± 10% and a photoperiod of 10: 14 h (L: D). Larval survival, larval development time, and pupal weight were recorded for each pest. The overall results of this work indicated that *B. carinata* has several species of insects associated with the crop in the southeastern U.S. The insect distribution on the plant canopy was

not uniform. The number of pods/plant is negatively impacted by defoliation during early stages, and *B. carinata* is a suitable host plant for *T. ni* and *P. xylostella*.

CHAPTER 1
INTRODUCTION

Brassica carinata (A. Braun) (Brassicales: Brassicaceae), commonly known as Ethiopian mustard or Abyssinian mustard is not found in uncultivated environments and likely originated from a cross between *B. oleracea* L. CABBAGE and *B. nigra* L. BLACK MUSTARD (Kassa, 2002; Wang and Freeling, 2013). It was originally grown in Ethiopia (Kassa, 2002). The leaves are suitable as food when boiled, and the processed seeds are reported to alleviate upset stomachs (Kassa, 2002). The interest in *B. carinata* in the United States is due to its potential as a biofuel for jet fuel production (Seepaul et al., 2019).

The use of biofuels in the jet fuel industry has been constrained by the high costs of production, limited availability, and the lack of policies associated with biofuels and aviation (Gegg et al., 2014). The Clean Air Act was passed in 1970 to decrease pollutants that were present in the air. In 2005, the United States passed the Energy Policy Act (Energy Policy Act of 2005; EPA, 2017), which amended the Clean Air Act. The Energy Independence and Security Act was designed to help the United States reach energy independence, energy security, increase energy use efficacy, and increase the production of renewable fuels (Energy Independence and Security Act, 2007). The Energy Policy Act helped to establish the Renewable Fuel Standard, which is a federal program that requires fuel used in transportation to contain a minimum amount of renewable fuel (EPA, 2016; 2017). By 2022, the Renewable Fuel Standard aims to have 36 billion gallons of renewable fuel, that has a reduction in greenhouse gasses, when compared to a 2005 petroleum baseline (Energy Independence and Security Act, 2007; EPA, 2016; 2017). The reduction in greenhouse gases, must also take into consideration the direct and significant indirect emissions (EPA, 2016).

The Southeast Partnership for Renewables from Carinata (SPARC) project is a collaborative effort to integrate *B. carinata* into southeastern United States cropping systems. SPARC is funded for July 15, 2017 to July 14, 2019, by the United States Department of Agriculture-National Institute of Food and Agriculture (USDA-NIFA, 2019). Several industry partners are working in collaboration with the SPARC project: Agrisoma Biosciences, Inc., Applied Research Associates, Commercial Aviation Alternative Fuels Initiative, and Glades Crop Care (SPARC, 2017a). The SPARC project focuses on *B. carinata* production in Alabama, Georgia, North Carolina, Tennessee, Mississippi, and Florida, because the environmental conditions of these states may provide a suitable climate for *B. carinata* cultivation as a winter crop (Seepaul et al., 2018). The objectives of the SPARC project are to explore the potential of *B. carinata* as an economic winter crop in the region and increase the production of a sustainable biofuel to meet the demand of the jet fuel industry (SPARC, 2017b). The project is multidisciplinary and there are teams developing improved feedstock, the use of the seed as a feedstock, development of supply chains, analysis of *B. carinata* agricultural and industrial processes, and increase of the production/optimization of *B. carinata* as a jet fuel (SPARC, 2017c). The SPARC project is developing *B. carinata* as a sustainable biofuel for aircraft in the United States through research and collaboration with industry partners (SPARC, 2017a, b, c). The SPARC project is a recent addition to the United States interests in renewable fuels.

The integration of *B. carinata* into southeastern United States cropping systems to produce biofuel demands the development of an Integrated Pest Management (IPM) program. The first steps for an IPM program should be to document the occurrence of insects associated with the crop, how suitable *B. carinata* is as an insect host plant, and the resulting loss from injury that pests can cause in the crop.

Based on these considerations the hypotheses and corresponding objectives of this work are:

Hypotheses

1. There are no species of insects that feed on *B. carinata* cultivated during the winter/spring crop season in the southeastern United States.
2. Insects that feed on *B. carinata* have a uniform distribution on the plant canopy.
3. Defoliation during different crop stages of *B. carinata* does not impact the number of seeds per pod, thousand seed weight, and the number of pods per plant.
4. *Brassica carinata* is not a suitable host for lepidopteran-pests.

Objectives

1. Document insect pests associated with *B. carinata* genotypes in the Florida Panhandle during different phenological stages.
2. Determine insect pest distribution within the *B. carinata* canopy.
3. Quantify the impact of defoliation during *B. carinata* vegetative and reproductive stages on the number of seeds per pod, thousand seed weight, and the number of pods per plant.
4. Assess the effect of *B. carinata* genotypes on life history traits of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae).

CHAPTER 2
PESTS ASSOCIATED WITH *BRASSICA CARINATA*

The documentation of pest species associated with *B. carinata* and possible differences in pest susceptibility among genotypes are needed to establish an integrated pest management (IPM) program for this crop. *Brassica carinata* has been reported to be resitant to insects and diseases (Malik, 1990; Getinet et al., 1996). One of the explanations for this resisant is the presence of secondary compounds that benefit the plant by providing a chemical defense against herbivores (Halkier and Gershenzon, 2006; Speight et al., 2008; Klowden, 2013). Secondary compounds can be important in plant defense by interfering with insect feeding, attracting insect predators (Stephenson, 1982) or as plant signaling compounds that affect the development of other organs, such as haustoria (Keyes et al., 2000). Members of Brassicaceae are known to synthesize 30 to 40 different glucosinolates, synthesized from amino acids and glucose (Halkier and Gershenzon, 2006).

The concentration of glucosinolates in *Brassica* species can change throughout plant stages, plant tissues, and plant canopy position (Bellostas et al., 2004; Gols et al., 2018). Availability of nutrients such as sulfur and nitrogen influence the presence of glucosinolates in canola (Zhao et al., 1993), and herbivory was found to cause a slight change in glucosinolate content in *B. oleracea* (Gols et al., 2018). In *B. nigra*, *B. juncea*, and *B. carinata* the highest levels of glucosinolates were found to be in the reproductive tissues, but glucosinolate concentration throughout the various parts of the plant and stages is dynamic (Bellostas et al., 2004). In *Brassica napus* L. CANOLA, glucosinalates were reported in both the leaf and stem tissues, but the glucosinolate content can vary among leaves of different age and positions on the plant (Porter et al., 1991).

There are few reports of glucosinolates and other secondary compounds specific to *B. carinata*, and it is difficult to determine a possible role in influence the pests feeding on this crop, as well the influence of the pest distribution on the plant canopy. However, some insects are able to overcome plant defenses because they have enzymes that can metabolized various secondary compounds, such as glucosinolates (Halkier and Gershenzon, 2006; Beran et al., 2014). According to Beran et al. (2014), glucosinolates present within *Brassica* species are not normally detrimental to several species of pests. In some cases, glucosinolates can trigger positive olfactory and gustatory responses in insects seeking a suitable host for consumption or reproduction (Speight et al., 2008; Klowden, 2013). These specialist insect herbivores have evolved a specialization on *Brassica* species and are stimulated to feed by glucosinolates. Such insects include *Phyllotreta* species (Coleoptera: Chrysomelidae) and *P. xylostella* (Thorsteinson, 1953; Lamb 1984; Loon et al., 2002; Halkier and Gershenzon, 2006). The secondary compounds can be also a relevant factor for pest canopy distribution (Speight et al., 2008). For example, *Mamestra configurata* Walker prefers the upper canopy of *Brassica* species (Ulmer, 2002). Ang et al. (2014) found that *P. xylostella* neonates had a preference for feeding on the youngest leaves of cabbage. In the same study, it was also reported that *P. xylostella* adults deposited more of their eggs on younger leaves than the older leaves. Based on these considerations, the hypotheses and objectives of this chapter are:

Hypotheses

1. There are no species of insects that feed on *B. carinata* cultivated during the winter/spring crop season in the southeastern United States.
2. Insects that feed on *B. carinata* have a uniform distribution on the plant canopy.

Objectives

1. Document insect pests associated with *B. carinata* genotypes in the Florida Panhandle during different phenological stages.
2. Determine insect pest distribution within the *B. carinata* canopy.

Materials and Methods

The survey of pests associated with *B. carinata* in the Florida Panhandle was performed at the West Florida Research and Education Center, Jay, FL. The experimental area was cultivated during the winter/spring crop seasons of 2017/2018 (30.776241˚lat, -87.135735˚long) and 2018/2019 (30.778208 ˚lat, -87.148315˚long) following the agronomic recommendations for canola in the southeastern U.S. (Seepaul et al., 2016). Planting dates were as follows: the 2017/2018 experimental area was planted on November 16, 2017 and the 2018/2019 experimental area was planted on December 19, 2018. Sixteen genotypes of *B. carinata* were evaluated in 2017/2018: AX17001, AX17002, AX17003, AX17004, AX17005, AX17006, AX17007, AX17008, AX17009, AX17010, AX17011, AX17012, AX17013, AX17014, AX17015, and AX17016. The genotype AX17016 is currently the commercial cultivar “Avanza 641”. The same genotypes of *B. carinata* were cultivated during the 2018/2019 crop season, except AX17015. These genotypes were selected because they are currently being evaluated by the SPARC project for development of commercial cultivars. The study was performed using a randomized complete block design, with four replications. Plots were six rows by 7.62 meters and were planted using a cone planter at a 0.19 meter-row spacing. Each row had 0.814 grams of seed for a total of 0.325 grams seed per plot.

The occurrence and abundance of pests associated with genotypes of *B. carinata* were documented by non-destructive plant sampling. The plants were inspected for pest presence, number of pest insects, and the position of the pest on the plant canopy. Plant inspection was

conducted by visually inspecting the top of the plant and moving toward its base. One plant per plot was randomly selected from the four center rows for plant inspection. Each plant was divided into three canopy zones. Canopy zones were created by evenly dividing the plant with 6 or more leaves into three portions: upper, middle, and lower. Plants were inspected for the presence of pests between 7 a.m. and 12 p.m. The phenological stage of the crop was noted at the start of each sampling vegetative stage (after development of 9 or more true leaves - over 50% of plants had no open flowers or developed pods), flowering stage (over 50% of plants had flower buds formed), and pod development stage (over 50% of plants had pods formed) (Seepaul, R, *personal communication*). Aphid samples were submitted to the Florida Department of Agriculture and Consumer Services-Division of Plant Industry (FDACS- DPI, E2019-579-1) for species identification.

The difference of pest occurrence and abundance among *B. carinata* genotypes and plant stages were analyzed using analysis of variances (R Core Team, 2018). Genotype and plant stage were fixed variables and the number of pests was the response variable. Variables that were not significant were not treated separately. Crop seasons were treated separately. Pearson's Chi-square analysis was used to evaluate the distribution of pests within canopy zones (R Core Team, 2018). The percentage of relative abundance was calculated for each species by dividing the total number of a single species by the total number of all insect pest species and then multiplied by 100. Pest canopy position and number of pests were graphed as balloon plots (R Core Team, 2018). Data was not transformed. A p-value was tested at a 95% confidence interval.

Results

During the 2017/2018 crop season, the occurrence of pests associated with *B. carinata* was not significantly different across crop stages (p-value = 0.690). Similarly, the occurrence of pests among different genotypes of *B. carinata* was not significantly different (p-value = 0.660).

The pest species detected in *B. carinata* during the 2017/2018 crop season included *M. ochroloma* adults and larvae, *P. xylostella* larvae, *Pieris rapae* L. (Lepidoptera: Pieridae) larvae, *Diabrotica undecimpunctata* Barber (Coleoptera: Chrysomelidae) adults, and *Lipaphis pseudobrassicae* Davis (Hemiptera: Aphididae) adults and nymphs (Table 2-1).

During the 2018/2019 crop season, the occurrence of pests was significantly different across crop stages of *B. carinata* (p-value = 1.60×10^{-4}), but the occurrence of pests was not different across the 15 genotypes under study (p-value = 0.770). The pest species detected during the 2018/2019 crop season included *M. ochroloma* adults and larvae, *P. xylostella* larvae, *P. rapae* larvae, *L. pseudobrassicae* adults and nymphs (samples identified by FDACS- DPI, E2019-579-1), *Leptoglossus phyllopus* L. (Hemiptera: Coreidae) adults, and *Chloridea virescens* F. (Lepidoptera: Noctuidae) larvae (Table 2-1).

Adults and larvae of *Microtheca ochroloma* were observed during both crop seasons (Figures 2-1, 2-2, 2-4, and 2-5). During the 2017/2018 crop season, in the flowering (p-value = 0.174) and pod development (p-value = 0.174) stages, adults of *M. ochroloma* presented a uniform distribution on *B. carinata* canopy (Figures 2-2 and 2-3). Larvae of *M. ochroloma* was not uniformly distribution on plant canopy in the flowering (p-value = 2.26×10^{-6}) and pod development (p-value = 0.013) stages and were most often located in the lower plant canopy (Figures 2-2 and 2-3). During the 2018/2019 crop season, in the flowering stage, the distribution of larvae of this species was uniform on plant canopy (p-value = 0.14). However, during the pod development stage the canopy distribution of *M. ochroloma* larvae (p-value = 6.90×10^{-5}) and adults (p-value = 6.90×10^{-16}) were concentrated on the upper canopy (Figure 2-5).

Plutella xylostella larvae were uniformly distributed in the 2017/2018 pod development stage (p-value = 1), and in the 2018/2019 vegetative stages (p-value = 0.470). During the

flowering stage of 2017/2018 crop season, *P. xylostella* larvae was concentrated on the lower plant canopy (p-value = 0.030), and during the flowering stage of the 2018/2019 crop season the larvae was concentrated in the middle plant canopy (p-value = 2.3×10^{-10}). During the 2018/2019 crop season *P. xylostella* occurred most often on the middle plant canopy of *B. carinata* (p-value = 2.2×10^{-16}).

Adults and larvae of *Lipaphis pseudobrassicae* did not have a uniform canopy distribution on *B. carinata* (Figures 2-1, 2-2, 2-3, 2-4, and 2-5). During the 2017/2018 crop season, in the flowering (p-value = 5.97×10^{-14}) and pod development (p-value = 2.2×10^{-16}) stages, *L. pseudobrassicae* occurred most often on the lower plant canopy (Figures 2-1 and 2-2). The same was observed in the 2018/2019 crop season, during pod development stage (p-value = 2.2×10^{-16}). During the 2018/2019 vegetative (p-value = 2.2×10^{-16}) and flowering (p-value = 1.60×10^{-4}) stages, *L. pseudobrassicae* occurred most often in the upper canopy (Figures 2-3 and 2-4).

Pieris rapae larvae had a uniform canopy distribution in both 2017/2018 and 2018/2019 crop seasons, in the flowering (p-value = 0.307; p-value = 0.370) and pod development stages (p-value = 0.565; p-value = 0.1) (Figures 2-1, 2-2, 2-4, and 2-5).

The two larvae of *Chloridea virescens* larvae detected during the 2018/2019 crop season, in the pod development stage had a uniform canopy distribution (Figure 2-5).

Discussion

Several species of pests have been documented to feed on *Brassica* species in the southeastern U.S., including *P. xylostella* (Ramachandran et al., 2000; Loon et al., 2002), aphids (Hemiptera: Aphididae) (Reddy, 2017), and *Microtheca ochroloma* Stal (Coleoptera: Chrysomelidae) (Manrique et al., 2012). The present study was conducted during two winter/spring crop seasons of *B. carinata* in the Florida Panhandle, performing 15 pest

samplings. The results indicated the occurrence of *M. ochroloma, P. xylostella, P. rapae, D. undecimpunctata, L. pseudobrassicae, L. phyllopus,* and *C. virescens.* These findings indicate there are species of insects that can utilize *B. carinata* as a host in the southeastern United States, In addition, the 16 genotypes of *B. carinata,* currently under evaluation for the development of commercial cultivars did not present any source of plant resistance by nonpreference to the species of pests documented in the present study. *Microtheca ochroloma* is an economic pest of *Brassica* species in the southeast (Ameen, 1996; Balusu and Fadamiro, 2011; Agrisoma, 2017; Reddy, 2017). The preferred hosts of *M. ochroloma* are *B. rapa* and cabbage (Ameen, 1996; Balusu and Fadamiro, 2011). However, *M. ochroloma* can use other *Brassica* species as host plants (Ameen, 1996; Balusu and Fadamiro, 2011). During the winter/spring crop season multiple and overlapping generations of *M. ochroloma* can occur (Reddy, 2017). The present study indicated that *B. carinata* was a host plant for *M. ochroloma* and the pest was observed on all canopy zones.

Plutella xylostella is a major pest of plants of the *Brassica* genus worldwide, causing economic impact in North America, South America, Central America, Africa, Europe, and the Caribbean (Furlong et al., 2013; CABI, 2018). Twenty known wild and cultivated *Brassica* species have been reported as hosts for this pest, and hosts for this pest also include non-*Brassica* species members such as Malvaceae, Fabaceae, and Asteraceae (CABI, 2018). This species can have multiple and overlapping generations within a single growing season (Talekar and Shelton, 1993), and temperature and host availability define the density of *P. xylostella* populations (Ngowi et al., 2017). In both crop seasons in this study, this species was detected associated with *B. carinata*, and during the 2018/2019 crop season, this species occurred in all crop stages. The

samples performed during two crop seasons indicated that *P. xylostella* larvae did not have a uniform distribution on canopy zones, in the vegetative, flowering or pod development stages.

Aphididae species have also been reported as pests of *Brassica* species (Reddy, 2017). Damage to canola by aphids may include flower abortion and pod damage, negatively impacting yield and plant height (Reddy, 2017). The major aphid species documented in canola in the southeast are *Lipahis erysimi* Kaltenbach, *Brevicoryne brassicae* (L.), and *Myzus persicae* Sulzer (Reddy, 2017), and Mezgebe et al. (2018) reported *B. carinata* as suitable host for *B. brassicae.* However, none of these species were detected during the pest sampling in this study. *Lipaphis pseudobrassicae* was the species of aphids detected during the pest sampling and was the most common insect associated with *B. carinata* during both crop seasons.

No pattern of canopy distribution of *L. pseudobrassicae* was detected. According to Reddy (2017), canopy distribution of *L. pseudobrassicae* would be predominant on the upper canopy within the flowering portions of the *Brassica* plants. This pattern of aphid canopy distribution was observed during the 2018/2019 crop season, in both vegetative and flowering stages. However, during the 2017/2018 crop season, *L. pseudobrassicae* were most prevalent on the lower canopy of the plant. This lower canopy distribution followed the same distribution pattern reported by Sampaio et al. (2017) for *L. pseudobrassicae* when feeding on *B. oleracea.*

The least common pests detected in both the crop seasons were *P. rapae*, *D. undecimpunctata*, *L. phyllopus*, and *H. virescens*. In the U.S., *P. rapae* has been documented as a minor pest in canola and there are no reports documenting *P. rapae* as an economic pest (Bucur and Rosca, 2011). Based on the samplings performed in the present study, *P. rapae* was not abundant in *B. carinata* and is only expected to be a minor pest of *B. carinata* in the southeastern United States.

Diabrotica species are pests in corn (*Zea mays* L.) (Ma et al., 2009; Robert et al., 2017), peanut (*Arachis hypogaea* L.) (Barbercheck et al., 1995), sweet potato (*Ipomoea batatas* (L.) Lam.), and cucurbits, (Jackson et al., 2005). However, *Diabrotica* species have a large range of hosts, including several *Brassica* species such as canola, cabbage, and bok choy (Walsh, 2003). *Diabrotica undecimpunctata* was only found in the adult stage and in low numbers on *B. carinata* during the reproductive stage of the crop. Future pest survey in *B. carinata* should include destructive samplings of stems and roots to determine if *B. carinata* is a host for this pest.

Leptoglossus phyllopus is a minor polyphagous pest in Rutaceae (Henne et al., 2003), Asteraceae, Bignoniaceae, Cucurbitaceae, Lamiaceae, Malvaceae, Orobanchaceae, Onagraceae, Scrophulariaceae, Solanaceae and Fabaceae (Mitchell, 2006). In the present study *L. phyllopus* was found in low abundance and feeding on *B. carinata* during the pod development stage, in the 2018/2019 crop season.

Chloridea virescens was found once during the *B. carinata* pod development stage in 2018/2019 crop season. This is a polyphagous pest of field crops and has been reported feeding on *Brassica* species (Capinera, 2012).

There were differences in pest abundance during the two crop seasons. Differences in planting dates (2017/2018: November 16th, 2017 and 2018/2019: December 19th, 2018) resulted in differences in the overall temperature the crop was cultivated during the two crop seasons, which could have influenced the pest abundance (Table 2-2). These results are representative of the variation in the abiotic factors during the winter/spring crop season in southeastern U.S, which could influence the annual occurrence and abundance of pests in the region.

Besides the documentation of species of insects associated with *B. carinata*, the present study indicates the potential of this crop as a suitable source for early season pest infestation and its possible role as a nursery or trap crop on a temporal scale for summer crop pests in the region. This information may assist in predicting the seasonal abundance of pests within the landscape of the southeastern U.S. Species of pests that can be associated with *B. carinata* are expected to have multiple generations (Reddy, 2017). If following *B. carinata*, a field is immediately planted with a summer crop that is a host plant of the species previously listed, the summer crop could have a higher level of pest infestation than if the land had been fallow (Altieri et al., 1984). On the other hand, the increase of the natural enemy populations early in the summer crop season should also be considered and further evaluated. Lundgren and Fergen (2010) found that a cover crop, *Elymus trachycaulus* (Poales: Poaceae) could decrease a pest population by supporting natural enemies.

The goal of this work was to provide information that will contribute in the development of an Integrated Pest Management Program for *B. carinata* in the southeastern U.S. The documentation the occurrence of pests during the different phenological stages helps to design IPM strategies, providing insight into which pests are expected to be present and require management. Moreover, the documentation of canopy position of pests on *B. carinata* indicates where the producers should inspect their crop for pests, as well as could improve the chemical control applications and release of and biological control agents (Plouvier and Wajnberg, 2018).

Table 2-1. Occurrence and relative abundance of insects associated with *B. carinata* in the Florida Panhandle during the 2017/2018 and 2018/2019 winter/spring crop seasons, Jay, FL.

	Relative abundance[1] (%)		
	----------Crop stage----------		
Species of pest	**Vegetative[2]**	**Flowering[3]**	**Pod development[4]**
		2017/2018	
Pieris rapae	-	7.0	3.3
Microtheca ochroloma	-	10.8	6.2
Plutella xylostella	-	5.1	6.2
Diabrotica undecimpunctata	-	2.5	1.0
Lipaphis pseudobrassicae	-	74.5	82.4
		2018/2019	
Pieris rapae	0	0.6	0.9
Microtheca ochroloma	0	1.1	5.3
Plutella xylostella	0.6	57	48
Diabrotica undecimpunctata	0	0	0.2
Lipaphis pseudobrassicae	99	42	44
Leptoglossus phyllopus	0	0	1.1
Chloridea virescens	0	0	0.2

Relative abundance was calculated by dividing the total number of a single species by the total number of all pest insect species and then multiplied by 100.
Vegetative stage of *B. carinata* was over 50% of plants in experimental area have no open flowers or developed pods.
Flowering stage of *B. carinata* was over 50% of plants in experimental area have flower buds formed. Pod development stage of *B. carinata* was over 50% of plants in experimental area have pods formed.

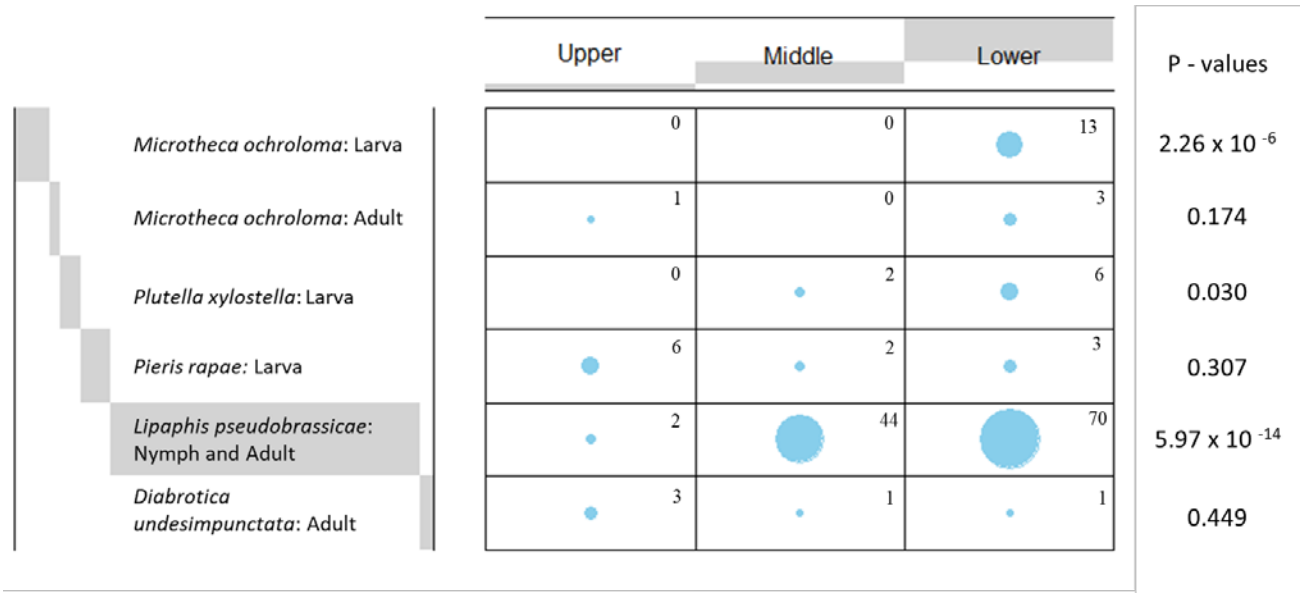

Figure 2-1. Distribution and frequency of species of insects on canopy zones of the *Brassica carinata* plant during the flowering stage in the 2017/2018 winter/spring crop season. Jay, FL. The number labels on the figure represent total number of insects within the sampling period. The p-value comes from the chi-square analysis comparing each species to against itself on canopy position.The data is displayed in proportion to each other on the balloon plot. The shading on the upper x-axis shows what proportion of the insects were found in that plant canopy zone. The shading on the y-axis shows what proportion of the insects were a species.

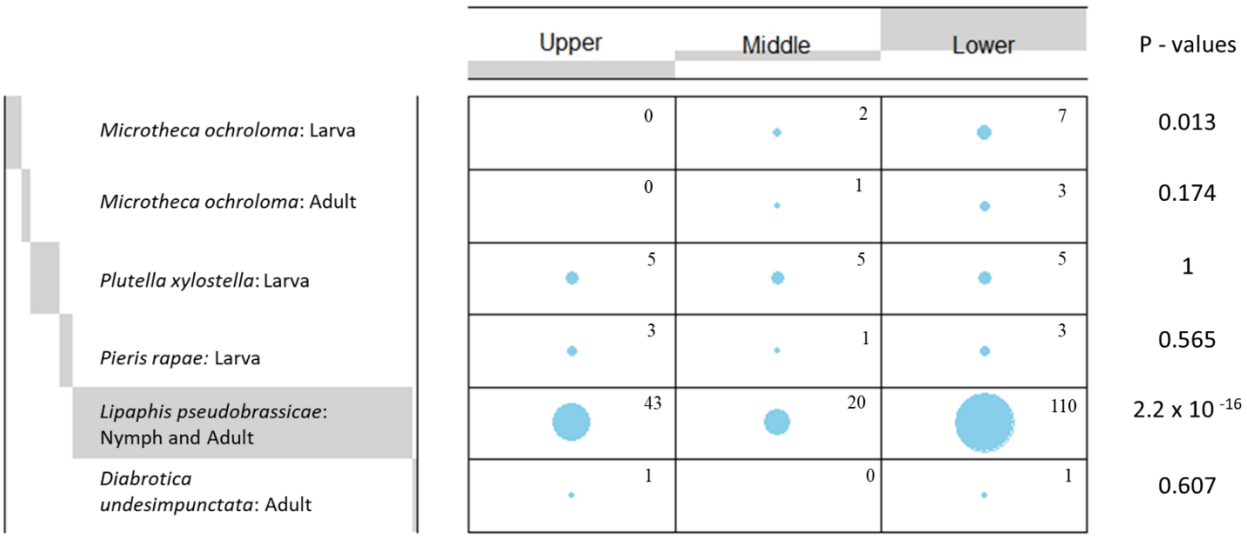

Figure 2-2. Distribution and frequency of species of insects on canopy zones of the *Brassica carinata* plant during the pod development stage in the 2017/2018 winter/spring crop season. Jay, FL. The number labels on the figure represent total number of insects within the sampling period. The p-value comes from the chi-square analysis comparing each species to against itself on canopy position. The data is displayed in proportion to each other on the balloon plot. The shading on the upper x-axis shows what proportion of the insects were found in that plant canopy zone. The shading on the y-axis shows what proportion of the insects were a species.

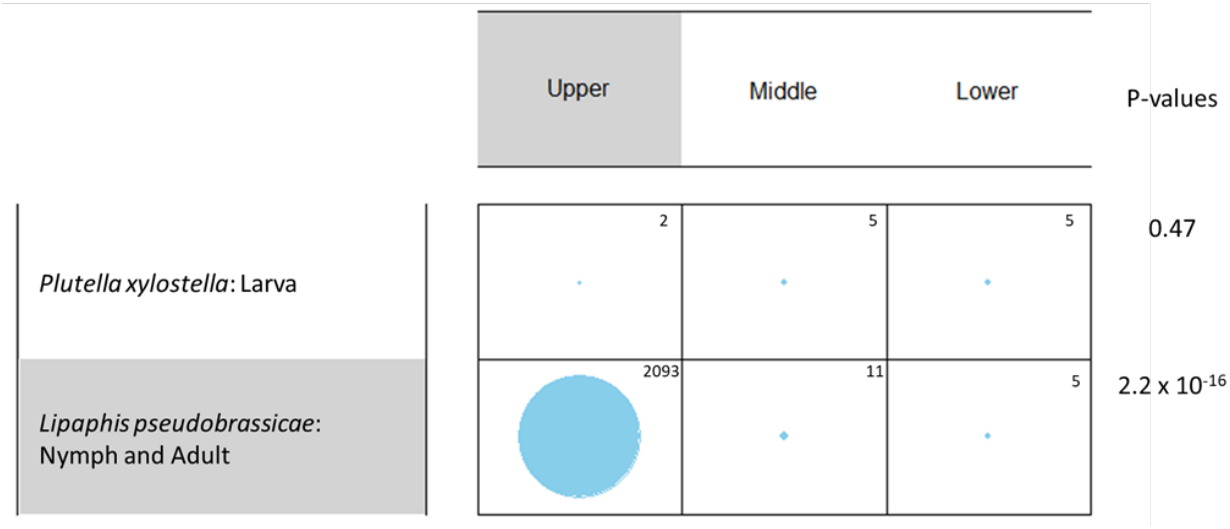

Figure 2-3. Distribution and frequency of species of insects on *Brassica carinata* plant canopy zones during the vegetative crop stage in the 2018/2019 winter/spring crop season Jay, FL. The number labels on the figure represent total number of insects within the sampling period. The p-value comes from the chi-square analysis comparing each species to against itself on canopy position. The data is displayed in proportion to each other on the balloon plot. The shading on the upper x-axis shows what proportion of the insects were found in that plant canopy zone. The shading on the y-axis shows what proportion of the insects were a species.

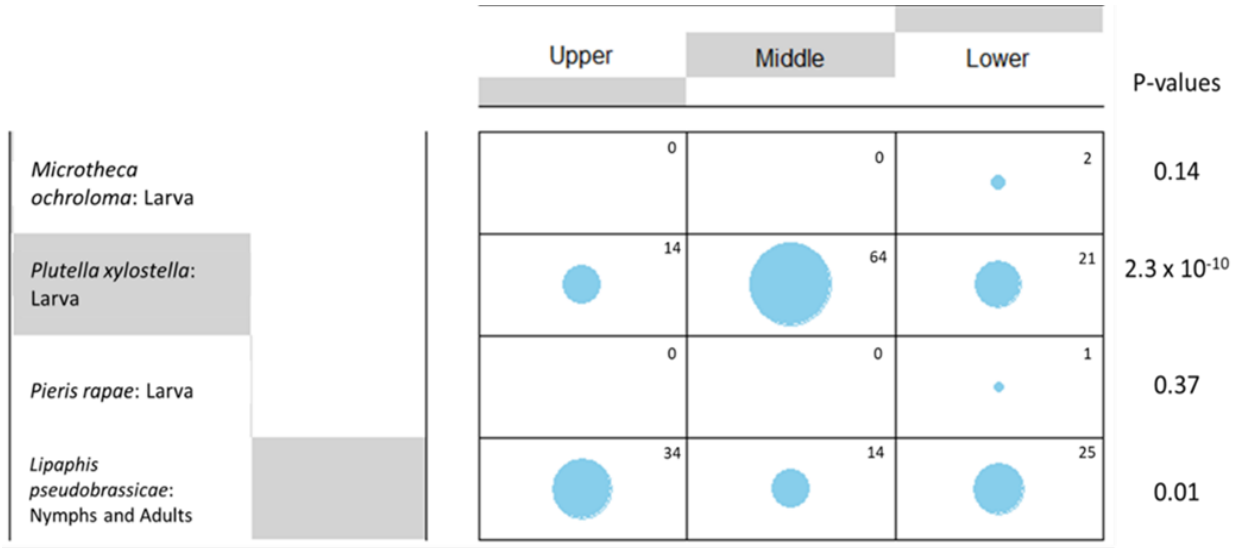

Figure 2-4. Distribution and frequency of species of insects on *Brassica carinata* plant canopy zones during the flowering crop stage in the 2018/2019 winter/spring crop season, Jay, FL. The number labels on the figure represent total number of insects within the sampling period. The p-value comes from the chi-square analysis comparing each species to against itself on canopy position. The data is displayed in proportion to each other on the balloon plot. The shading on the upper x-axis shows what proportion of the insects were found in that plant canopy zone. The shading on the y-axis shows what proportion of the insects were a species.

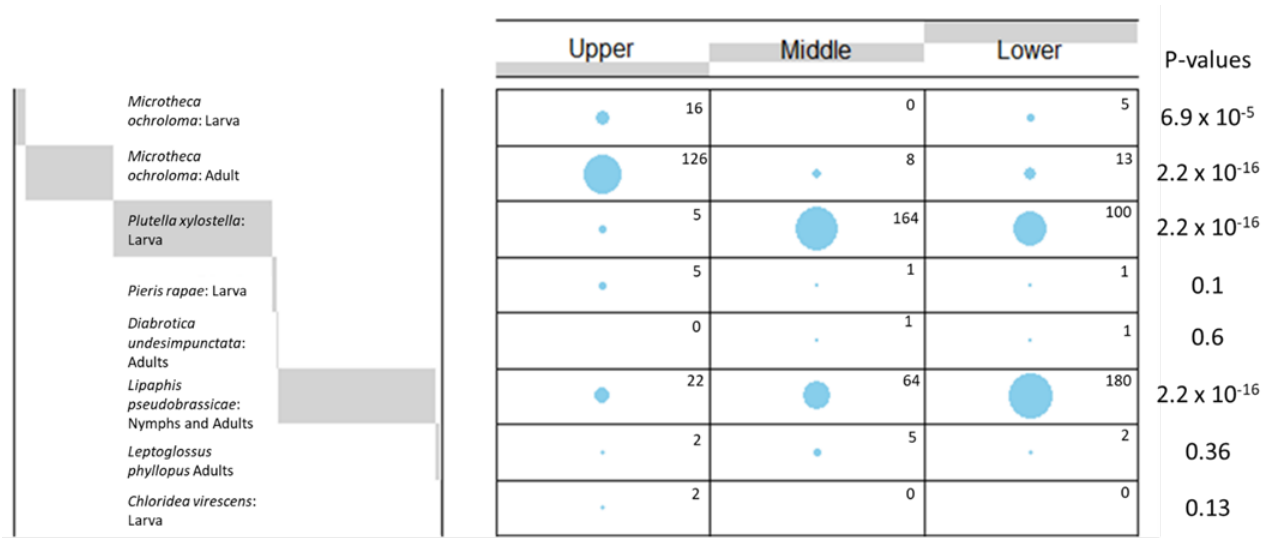

Figure 2-5. Distribution and frequency of species of insects on *Brassica carinata* plant canopy zones during the pod development crop stage in the 2018/2019 winter/spring crop season, Jay, FL. The number labels on the figure represent total number of insects within the sampling period. The p-value comes from the chi-square analysis comparing each species to against itself on canopy position. The data is displayed in proportion to each other on the balloon plot. The shading on the upper x-axis shows what proportion of the insects were found in that plant canopy zone. The shading on the y-axis shows what proportion of the insects were a species.

Table 2-2. Monthly average of temperature and standard deviation during the 2017/2018 and 2018/2019 winter/spring crop seasons. WFREC, Jay, FL.

	Temperature (C°)	**SD (±)**
	2017/2018	
November	15.24	3.88
December	11.3	5.99
January	7.3	6.63
February	17.35	4.03
March	15.13	4.07
April	17.55	2.73
May	23.67	1.25
	2018/2019	
November	13.08	5.51
December	12.20	4.86
January	10.31	5.04
February	16.18	3.79
March	14.54	4.62
April	18.51	2.91
May	24.03	2.08

CHAPTER 3
IMPACT OF DEFOLIATION ON *BRASSICA CARINATA* AND THE EFFECT ON LIFE HISTORY TRAITS OF LEPIDOPTERAN-PESTS

Defoliation can have a varying impact on crop yield (Pandey, 1983; Rajewski et al., 1991; Ramachandran et al., 2000; Lauer et al., 2004; Mccormick et al., 2013; Thrash, 2018). Studies with *B. napus* indicate that the impact of defoliation on seed production depends on the crop stage (Major et al., 1978; Ramachandran et al., 2000). Early defoliation in *B. napus* delays flowering and decreases the rate of stem growth (Mccormick et al., 2013). Defoliation in *B. napus* during flowering and vegetative stages can impact pod and seed development (Ramachandran et al., 2000). However, during the pod development stage, defoliation in *B. napus* has little to no effect on yield.

The development of cultivars of a novel crop, such a *B. carinata* should consider the level of resistance to herbivory and if the crop is suitable as a host plant (Thorsteinson, 1953; Talekar and Shelton, 1993; Zalucki et al., 2012; Furlong et al., 2013; Mezegebe et al., 2018; Fathipour et al., 2019). Experiments with canola have been performed to test cultivar resistance to the feeding of *P. xylostella* (Ulmer, 2002; Musser et al., 2005). Factors that aid in pest selection of plants can be olfactory (Loon et al., 2002; Halkier and Gershenzon, 2006; Klowden, 2013) or visual (Ulmer, 2002; Musser et al., 2005) cues. Similar studies have yet to be conducted with *B. carinata*.

The life history traits of pests that include parameters such as larval weight, pupal weight, adult longevity, and number of eggs produced can be influenced by insect food source (Golizadeh et al., 2009; Rivera-Vega et al., 2017). Insects may also have enzymes such as glutathione S-transferase (Yu, 2015), and cytochrome P450 (Yu, 2015) that are used to metabolize ingested secondary compounds of host plants (Klowden, 2013). Production of these enzymes to is metabolically expensive and therefore, can decrease insect fitness (Fournier, 2005;

Yu, 2015). However, some species have evolved to exploit the secondary compounds plants produce for defense which can act as attractants (Loon et al., 2002).

The life cycle of *P. xylostella* is greatly influenced by temperature and the number of generations per growing season can be highly variable (Ngowi et al., 2017). In addition, food sources can also influence *P. xylostella* fitness parameters (Golizadeh et al., 2009). Feeding on *Brassica* species can influence the development of *P. xylostella* because of the differences in the host quality and presence of secondary compounds (Thorsteinson, 1953; Mezegebe et al., 2018; Fathipour et al. 2019). The *Plutella xylostella* fecundity and lifespan were evaluated in a feeding study with five different *B. napus* cultivars. The shortest generation time for this species on canola was found to be eighteen days, while the longest was twenty-one days (Faithipour et al., 2019). Golizadeh et al. (2009) evaluated the life history of *P. xylostella* on cauliflower, (*B. oleracea* L.) cabbage cv 'Globemaster', cabbage cv 'Scarlet Ohara', kohlrabi, and canola. Canola was the least favorable of the five hosts (Golizadeh et al., 2009). In addition, the authors reported an increase in developmental time and larval mortality of *P. xylostella* was associated with canola.

Trichoplusia ni is a generalist pest reported on vegetables cultivated in greenhouses (Mason and Huber, 2002) and field crops (Nault and Speese, 2002). This pest has several hosts in North America, such as tomato (Mason and Huber, 2002; Nault and Speese, 2002), *Brassica* species (Mason and Huber, 2002; Rivera-Vega et al., 2017), cucumber, and pepper (Mason and Huber, 2002). However, the preferred hosts of *T. ni* are *Brassica* species (Mason and Huber, 2002; Nault and Speese, 2002; Rivera-Vega et al., 2017). The development time of *T. ni* is reduced when feeding on *Brassica* species compared with feeding on non-*Brassica* hosts (Rivera-Vega et al., 2017). In laboratory feeding studies with *Brassica* species, *T. ni* larvae took

fifteen days to reach pupation while larvae feeding on the tomato diet took twenty-three days to reach pupation (Rivera-Vega et al., 2017). Pest feeding preference for *B. carinata* genotypes and cultivars may influence plant selection.

In order to develop an IPM program for this crop, the life history traits of pests feeding on *B. carinata* and the impact of defoliation during different phenological stages needs to be documented. Based on these considerations the hypotheses and objectives of this chapter were:

Hypotheses

1. Defoliation during different crop stages of *B. carinata* does not impact the number of seeds per pod, thousand seed weight, and the number of pods per plant.
2. *Brassica carinata* is not a suitable host plant for lepidopteran-pests.

Objectives

1. Quantify the impact of *B. carinata* defoliation during vegetative and reproductive stages on number of seeds per pod, thousand seed weight, and the number of pods per plant.
2. Assess the effect of *B. carinata* genotypes on life history traits of *P. xylostella* and *T. ni.*

Materials and Methods

Defoliation Study

The impact of defoliation in *B. carinata* were estimated in a field study at the West Florida Research Education Center, Jay, FL, with the commercial cultivar "Avanza64". The experiment was conducted during the two winter/spring crop seasons and was arranged as a split-plot design, with four replications.

The 2017/2018 (30.776200˚lat, -87.137820˚long) and 2018/2019 (30.778051˚lat, -87.148422˚long) experimental areas were established following the agronomic recommendations for canola in the southeastern United States (Seepaul et al., 2016). The experimental area planted on November 2017 was killed by frost. The second planting of the 2017/2018 experimental area was on February 22, 2018 and harvested on June 28, 2018. Each replication was a plot with 38.1

meters by 10.7 meters. Row spacing was 0.381 meters, with eleven rows. The first and eleventh rows were the borders of each plot. The 2018/2019 experimental area was planted on December 6, 2018 and harvested on June 3, 2019. Each replication was 73.2 meters by 9.1 meters with 0.381 meters of row spacing, and each plot had fifteen rows. The first and the fifteenth rows were the borders of each plot. One application of pyrethroid was performed one time during each crop season in the experimental area to avoid natural infestation of defoliators insects.

Simulated defoliation was achieved by removing leaves by hand (Ramachandran et al., 2000; Batistela et al., 2012) at three crop phenological stages: vegetative (50% of plants had nine or more true leaves), flowering (50% of plants had between 20-30% of flowers open) and pod development (50% of plants pods were at mature size) (Seepaul, R, *personal communication*).

During the 2017/2018 crop season five levels (0%, 5%, 25%, 50%, and 100%) of one-time removal artificial defoliation was performed. During the 2018/2019 crop season, based on the analysis of the data of previous year, the levels of defoliation were adjusted to 0%, 50%, 75%, 90%, and 100%, and two treatments of continuous defoliation were also included. The 50% and 100% continuous defoliation treatments started at vegetative, flowering, and pod developments stages. The continuous defoliation treatments were implemented by returning to the row each week and removing new leaf growth.

In the 2017/2018 crop season, the defoliation study was performed with five randomly selected plants in the same row. In order to obtain sufficient seed for yield component analyses, during the 2018/2019 crop season fifteen plants per plot were randomly selected and defoliated. Plants selected on each row had at least one non-treated plant between them. The number of leaves removed was as follows: 0%-no leaves removed; 5%-every 20th leaf removed; 25%-every 4th leaf removed; 50%-every other leaf removed; 75%-every three leaves removed, and

the fourth leaf left; 90%-removal of nine out of every ten leaves; and 100%-all leaves removed. Plants were labeled with flagging tape to identify their defoliation level.

When plants reached full development, five plants were harvested. The number of pods per plant and the average number of seeds per pod of each plant was counted. The number of the seeds per pod was determined by sampling three pods per plant and count the number of seeds per pod. Thousand seed weight was determined by weighing 100 seeds and multiplying the weight by 10. The moisture content was estimated with a plant moisture tester (GEHAKA AGRI, Moisture Tester G600), and the seed weight was adjusted to 9.03% moisture content.

Feeding Study

Feeding studies with lepidopteran-pests were performed under controlled conditions in growth chambers at the West Florida Research and Education Center, Jay, FL. Two feeding experiments were conducted in a factorial design with *T. ni* and *P. xylostella* feeding on four *B. carinata* genotypes (AX17001, AX17008, AX17012, and AX17016, 'Avanza64') in a randomized complete block design. The three growth chambers were each treated as a replication. Three plants of each genotype of *B. carinata* were individually infested with neonates of *T. ni* and *P. xylostella*, for a total of three plants infested/genotype/growth chamber.

Eggs of *T. ni* and *P. xylostella* were obtained from Benzon Inc. (Carlisle, PA). The selection of genotypes of *B. carinata* was based on the seed glucosinolate content (Agrisoma ®) as following: AX17001 (63.3 µmol/g of seed), AX17016 (78.6 µmol/g of seed) AX17008 AX17012 (82.9 µmol/g of seed), and (105.3 µmol/g of seed). The plants were cultivated in two-gallon pots, with Sun Gro-Professional Growing Mix (Agawam, MA) under greenhouse conditions until six weeks of age to ensure that there was enough leaf development during the vegetative stage. One week after emergence, plants were fertilized with Miracle-Gro Tomato

Plant Food (Marysville, OH) (water soluble). The seeds were not treated, and pesticides were not applied to the plants.

The infestation of *P. xylostella* neonates was performed on each genotype of *B. carinata* using a fine touch brush. Due the small size of *P. xylostella* larvae, three neonates were infested per leaf, for a total of three infested leaves per plant. Each leaf was covered with a small (7.62 centimeters by 15.2 centimeters) mesh pollination bags (Delnet Pollination Bags, Middletown, DE) and tied with flagging tape on the base of the leaf. Due to the larger size of later instars, the infestation with neonates of *T. ni.* on the four genotypes of *B. carinata* were performed with two larvae/plant. After larval infestation, the plants were completely covered by a mesh pollination bag (96.52 centimeters by 167.6 centimeters) (Delnet Pollination Bags, Middletown, DE) and tied with flagging tape on the base of the pot. A set of 12 plants (four genotypes, with three plants, infested with one pest species) were placed in each growth chamber, set at 19.5 ± 2 ºC constant temperature, with a relative humidity 70 ± 10%, and a photoperiod of 10:14 h (L:D).

In both feeding experiments, the success of neonate infestation was evaluated on each plant twenty-four hours after infestation. Neonates deceased in this period were replaced. Larval survival and development were monitored until pupation. Once pupation was achieved, larvae were removed when the pupa was completely sclerotized. Before weighing, pupae of *P. xylostella* were removed from the cocoon using forceps to hold the cocoon and a fine touch brush to pull the cocoon away. The mean of the larval development time, pupal development time, pupal weight, and adult longevity for each species feeding on the four genotypes of *B. carinata* was determined.

Data Analysis

An ANOVA was performed to test for differences of number of the pods per plant, number of seeds per pod, and thousand seed weight under different defoliation levels at different

crop stages of *B. carinata*. Mean differences were determined using the Tukey-Kramer adjustment ($\alpha = 0.05$). Linear regression analyses were performed to determine the relationship between level of defoliation and the number of pods per plant and the number of seeds per pod. The data analysis program used was "R" (R Core Team, 2018). A p-value was evaluated at the 95% confidence interval. The data collected during the 2017/2018 and 2018/2019 crop seasons were treated as separate experiments.

An ANOVA was performed to test differences of pupal weight, larval development time, and percentage of mortality for *P. xylostella* and *T. ni* feeding in the four genotypes of *B. carinata*. A p-value was considered significant if it was at the 95% confidence interval.

Results

Defoliation Study

During the 2017/2018 crop season, the number of seeds per pod did not differ in plants subjected to defoliation during the vegetative (p-value = 0.216), flowering (p-value = 0.078), and pod development stages (p-value = 0.094). Similarly, during the 2018/2019 crop season, the number of seeds per pod was not different in plants subjected to defoliation at the vegetative (p-value = 0.0.313), flowering (p-value = 0.518), and pod development stages (p-value = 0.096). In the 2018/2019 crop season the thousand seed weight was estimated and was not different among defoliation levels at vegetative (p-value = 0.116), flowering (p-value = 0.945), and pod development (p-value = 0.548) stages.

At the vegetative stage, the linear relationship between defoliation and number of pods per plant of *B. carinata* was significant in both the 2017/2018 (p-value = 3.19×10^{-6}, $R^2 = 0.23$) and the 2018/2019 (p-value = 1.03×10^{-9}, $R^2 = 0.31$) crop seasons (Table 3-1). The reduction in number of pods per defoliation unit occurred at a rate of 0.86 and 0.96, respectively (Table 3-1). At the flowering crop stage, the linear relationship between defoliation and the number of pods

per plant was also significant in both the 2017/2018 (p-value = 0.0002, R^2 = 0.14) and the 2018/2019 (p-value = 0.0024, R^2 = 0.12) crop seasons (Table 3-1). The reduction in the number of pods per defoliation unit occurred at a rate of 0.54 and 0.58, respectively (Table 3-1). During the 2018/2019 crop season the relationship between continuous defoliation, starting at vegetative or flowering stages presented similar results. The number of pods per plant of *B. carinata* had a significant linear relationship with defoliation in plants at the vegetative (p-value = 3.12×10^{-7}, R^2 = 0.37) and flowering stages (p-value = 5.23×10^{-8}, R^2 = 0.39), with a rate of reduction of 0.89 and 0.71 number of pods/plant, respectivly (Table 3-1)

The mean number of the pods by defoliation levels at vegetative stage were separated by using Tukey's test, and indicated a significantly difference in the number of pods per plant, in both the 2017/2018 (p-value = 8.76×10^{-5}) and the 2018/2019 (p-value = 1.9×10^{-7}) crop seasons. During the 2017/2018 crop season, at the vegetative stage, 100% defoliation significantly impact number of the pods/plant when compared with of number of the pods of plants submitted to 0% (p-value = 6.4×10^{-5}), and 5% p-value = 9.9×10^{-5}. During the 2018/2019 crop season, at vegetative stage, the number of the pods per plant subjected to the defoliation level of 50% were significantly different than 100% (p-value = 0.013) and 90% (p-value = 0.039), but not different from 75% (p-value = 0.885). Similarly, impact of defoliation was significantly different in both 2017/2018 (p-value = 2.3×10^{-4}) and the 2018/2019 (p-value = 3.9×10^{-5}) crop seasons at flowering stage. During the 2017/2018 crop season, at the flowering stage, plant submitted to 100% defoliation were significantly different from defoliation levels of 0% (p-value = 7.2×10^{-4}) and 5% (p-value = 0.0364). During the 2018/2019 crop season, at the flowering stage plant submitted to 0% defoliation were significantly different from defoliation levels of 50% (p-value = 0.097), 75% (p-value = 0.005), 90% (p-value = 2.3×10^{-4}), and 100%

(p-value = 0.04). Overall, the impact of defoliation at vegetative and flowering stages of *B. carinata* was observed above 50% of defoliation (Figures 3-1 and 3-2).

At the pod development crop stage, in both the 2017/2018 (p-value = 0.120, R^2 = 0.02) and the 2018/2019 (p-value = 0.10, R^2 = 0.02) crop seasons, there was no linear relationship between defoliation level and number of pods per plant (Table 3-1), and defoliation at pod development stage did not impact number of the pods/plant (Figure 3-2).

Feeding Study

Overall, the life history traits of *P. xylostella* (p-value = 0.879) and *T. ni* (p-value = 0.148) were not different when the larvae feed on the four tested genotypes of *B. carinata.* The larval development time of *P. xylostella* feeding on different genotypes of *B. carinata* was 7.6 days (SD ± 0.47), while the *T. ni* larval development time in different genotypes was 19.44 days (SD ± 0.164) (Table 3-2). Larval mortality of *P. xylostella* during the feeding study was 40.7%. The larval mortality of T. *ni* was 2.1% (Table 3-2). Pupal weight of *P. xylostella* (p-value = 0.761) was not different when larvae fed on different genotypes of *B. carinata*. Similarly, T. *ni* (p-value = 0.696) presented the same pupal weight feeding in different genotypes. Pupal weight of *P. xylostella* and *T. ni* was 8.8 mg (SD ± 1.29) and 229.8 mg (SD ± 24.46), respectively (Table 3-2).

Discussion

The number of seeds per pod of *B. carinata* was not different among the levels of defoliation either in the 2017/2018 or in the 2018/2019 crop seasons. Thousand seed weight data was not recovered for the 2017/2018 crop season, because most of the seeds were moldy and there were not enough seeds in some plants to acquire the thousand seed weight. In the 2018/2019 crop season, the number of plants subjected to each defoliation treatment was

increased to 15 plants/treatment/plot in order to have enough seeds to estimate thousand seed weight. Similar to the number of seeds per pod, thousand seed weight among defoliation treatments was not significantly different. A possible explanation for the constant number of the seeds per pod and thousand seed weight in plants subjected to different levels of defoliation could be related to how much nutrition the seeds receive from the pod. Major et al. (1978) reported in *B. napus* that plant tissues allocate resources to different portions of the plant. The lower leaves of canola contribute to the photosynthates that are sent to the roots, while the upper leaves and stems contribute photosynthates to the pods and seeds. The pods in *B. napus* are also able to photosynthesize and retain those photosynthates. The authors also reported that in canola, pods do not transport assimilates outside of the pod, but they do serve as a sink for assimilates from the upper leaves and stems. In addition, King et al. (1997) suggested that in canola the pod wall contributes to the seed development with carbohydrates.

In some crops such as sorghum *Sorghum bicolor* (L.) Moench (Rajewski et al., 1991), soybean *Glycine max* (L.) Merr. (Thrash, 2018), and cowpea *Vigna unguiculate* (L.) Walp. (Pandey, 1983), defoliation can reduce seed yield at vegetative and flowering crop stages (Pandey, 1983; Rajewski et al., 1991). In addition, defoliation can influence the time a crop remains in a phenological stage (Rajewski et al., 1991). In *B. carinata*, our findings imply that the crop is only impacted by levels of defoliation over 50%, which agrees with results from studies with canola (Malik, 1990; Getinet et al., 1996; Ramachandran et al., 2000; Mccormick et al., 2013). The results of the present study indicated that *B. carinata* is tolerant of low levels of defoliation during the vegetative and flowering stages, and at the pod development stage is not impacted by defoliation. The defoliation of *B. carinata* during the vegetative and flowering stages might have caused the plant to allocate more resources into the vegetative tissues than it

would have without a defoliation event, similar to what is reported in canola (Ramachandran et al., 2000).

The development of economic injury levels for pest management decisions represents a key component for an IPM program. The results from the defoliation study represents the first step for the developing an economic injury level for insects that cause defoliation in *Brassica carinata*. In the economic injury level equation, plant injury and the consequent yield loss is often difficult to be determined in field conditions (Pedigo et al., 1986; Higley and Pedigo 1997; Pedigo and Rice, 2009). In the present study, we found that defoliation resulted in pod reduction. Based on the response of *B. carinata* to defoliation, a plant that produces fewer pods, even with a constant number of seeds per pod, would produce fewer seeds, and consequently less oil. A decrease in oil would negatively impact the value of the crop because commodity value is determined by the amount of oil produced (Seepaul et al., 2019). Based on the regression analyses, the relationship between unit of defoliation and the number of pods per plant was estimated (Pedigo et al., 1986; Higley and Pedigo 1997). At the vegetative stage, the reduction in the number of the pods per unit of defoliation was estimated to be around one pod per plant (b = -0.86 and -0.96). At the flowering stage, the reduction in the number of pods per plant per unit of defoliation was estimated to be half pod per plant (b = -0.54, and -0.58).

Feeding studies were performed to document the life table history traits of *P. xylostella* and *T. ni* feeding on *B. carinata*. The feeding study was performed on plants because preliminary studies with leaves placed in Petri dishes demonstrated that leaf deterioration happens quickly and compromises the study (Baldwin et al., unpublished data). Based on the results of the feeding study, it can be said that *P. xylostella* and *T. ni* can survive and develop on the four tested genotypes of *B. carinata*. The selection of these genotypes was based on a range of

glucosinolate content from 63.3 µmol/g to 105.3 µmol/g of seed. However, the glucosinolate content in the leaves could be vastly less than the content in the seeds, so the insects may not have been exposed to levels of glucosinolates that would significantly impact their development among the genotypes. Future feeding studies should include the determination of the glucosinolate content in the leaves for different genotypes. In canola, glucosinolate content was found to be variable throughout plant structure (Velasco et al., 2008; Bhandari, et al., 2015). In addition, the stage of the plant should be also considered when evaluating life table traits of pests feeding in different genotypes. The present study was performed with plants in vegetative stage. Bellostas et al. (2004) found that in the later stages of *B. carinata* development, the reproductive tissues had the highest concentration of glucosinolates.

Overall, the present study indicates that *B. carinata* when subjected to defoliation has a constant number and weight of the seeds per pod, and the number of the pods decreased. During the vegetative and flowering stages plants of *B. carinata* were more sensitive to defoliation and have a reduction rate of one and half pod per unit of defoliation, respectively. The results also indicated that *B. carinata* is a suitable host plant for *P. xylostella* and *T. ni*. Future studies should document the potential of defoliation of pests associated with *B. carinata*, such as *P. xylostella* and, *T. ni* for the development of economic injury levels.

Table 3-1. Linear regression equations for the relationship between pods/plant of *Brassica carinata* and defoliation levels, by crop phenological stage during the 2017/2018 and 2018/2019 crop seasons. WFREC, Jay, FL.

	Regression Equation[a] ($\hat{y}^b = a^c + b^d x^e$)	**Standard Error**			
Crop phenological stage		**Slope**	**Intercept**	**P- value**	**R^2**
		2017/2018			
Vegetative	$\hat{y}$ = 104.49 - 0.86x	0.143	8.01	3.19 x 10^{-6}*	0.23
Flowering	$\hat{y}$ = 103.68 - 0.54x	0.15	6.65	0.0002*	0.14
Pod development	$\hat{y}$ = 82.76 - 0.18x	0.11	5.98	0.12 n.s.	0.02
		2018/2019			
Vegetative	$\hat{y}$ = 150.29 - 0.96x	0.14	10.39	1.03 x 10^{-9}*	0.31
Flowering	$\hat{y}$ = 139.82 - 0.58x	0.15	11.08	0.0024*	0.12
Pod development	$\hat{y}$ = 191.05 + 0.47x	0.36	26.32	0.10 n.s.	0.02
		2018/2019- Continuous defoliation			
Vegetative	$\hat{y}$ = 125.73 -0.89x	0.15	7.66	3.12 x 10^{-7}*	0.37
Flowering	$\hat{y}$ = 147.15 - 0.71x	0.11	7.39	5.23 x10^{-8}*	0.39
Pod development	$\hat{y}$ = 187.40 + 0.16x	0.29	19.86	0.583 n.s.	-0.01

2017/2018 levels of defoliation: 0%, 5%, 25%, 50%, and 100 %, and 2018/2019 levels of defoliation: 0%, 50%, 75%, 90%, 100%; and 50% and 100% continuous defoliation.

* indicates significant value, while n.s. indicates not significant value.

$\hat{y}$ = pod/plant

a = intercept

b = slope

x = defoliation %

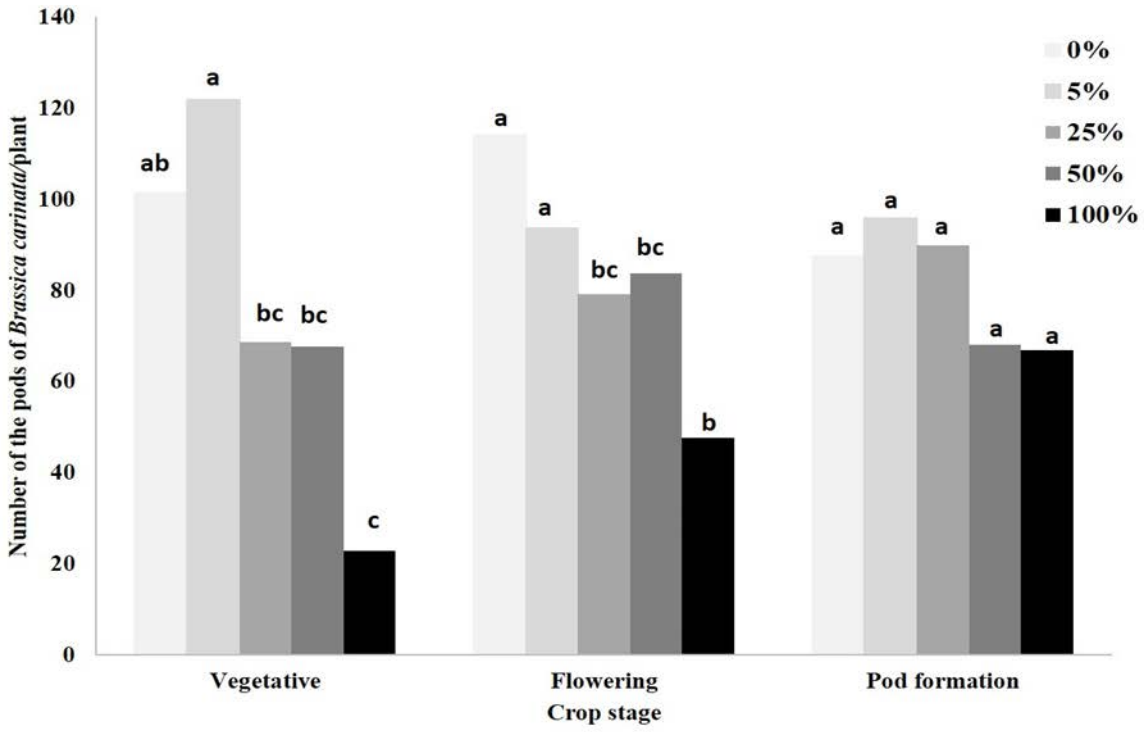

Figure 3-1. Number of pods per plant of *Brassica carinata* under different levels of defoliation. 2017/2018 crop season WFREC, Jay, FL. Mean number of pods/plant with the same letter in each crop phenological stage are not significantly different, Tukey's mean separation test, p-value ≤ 0.05. Defoliation levels were tested within their crop stage.

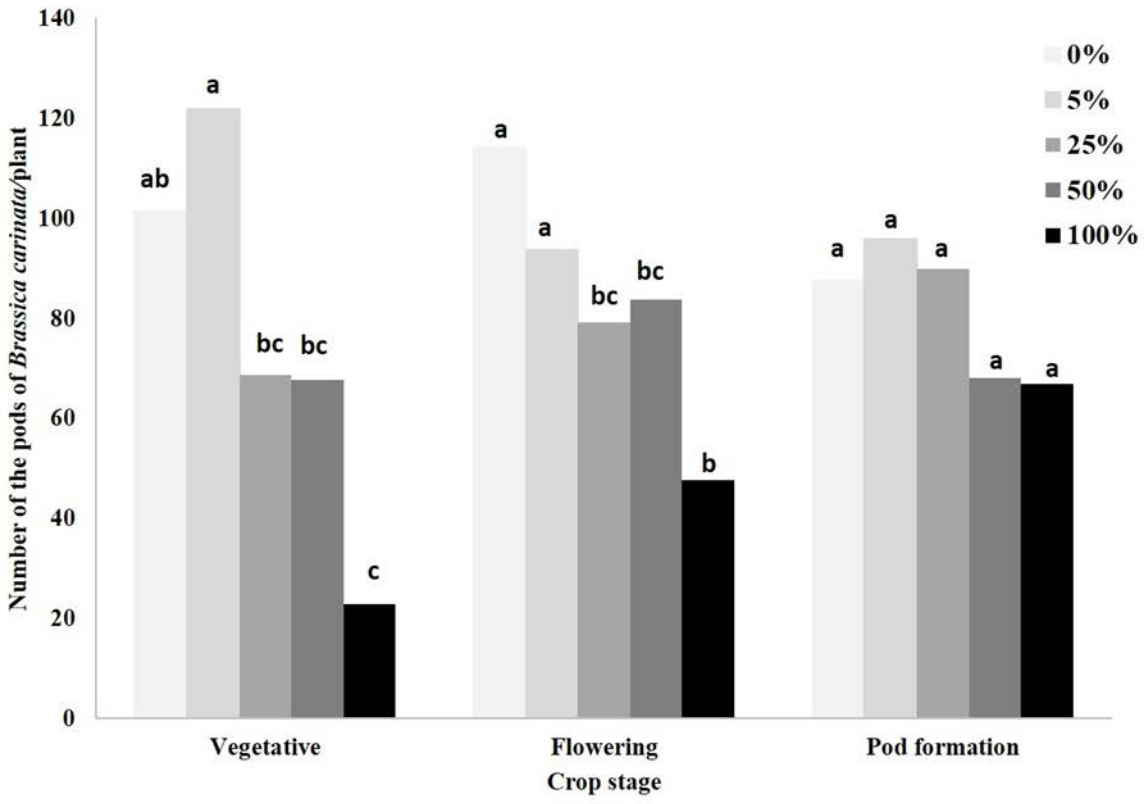

Figure 3-2. Number of pods per plant of *Brassica carinata* under different levels of defoliation. 2018/2019 crop season WFREC, Jay, FL. Mean number of pods/plant with the same letter in each crop phenological stage are not significantly different, Tukey's mean separation test, p-value ≤ 0.05. Defoliation levels were tested within their crop stage.

Table 3-2. Average of pupal weight, larval development time, and mortality of *Plutella xylostella* (n = 216) and *Trichoplusia ni* (n = 48) feeding on *Brassica carinata* WFREC, Jay, FL.

Species	**Pupal weight (mg)**	**SD (±)**	**Larval development time (days)**	**SD (±)**	**Larval mortality (%)**	**SD (±)**
P. xylostella	8.8	1.29	7.6	0.47	40.7	13.1
T. ni	229.8	24.46	19.44	0.164	2.1	5.89

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BIOGRAPHICAL SKETCH

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