Abstract

Brassica carinata has the potential to become an economical biofuel winter crop in the Southeast United States. An IPM program is needed to provide management recommendations for *B. carinata* in the region. This study serves as the first steps in the developing IPM tactics documenting pest occurrence, pest position within the canopy, and the impact of defoliation on *B. carinata* yield. The study was performed in Jay, FL, during the 2017/2018 and 2018/2019 winter/spring crop seasons. Pest species in *B. carinata* were documented by plant inspection within 16 genotypes of *B. carinata*, and the presence of insect pests in three canopy zones (upper, medium, and lower canopy) was documented. The defoliation impact on *B. carinata* was evaluated by artificial defoliation. Five levels of defoliation (2017/2018 crop season: 0%, 5%, 25%, 50%, and 100%; 2018/2019 crop season: 0%, 50%, 75%, 90%, and 100%) were artificially applied during vegetative, flowering, and pod formation stages of the commercial cultivar "Avanza64". During the 2018/2019 crop season, two experiments were performed, a one-time defoliation event and continuous defoliation. The plants were hand harvested and the average number of pods per plants, seeds per pod, thousand seed weight, and yield were estimated and correlated with defoliation levels. Results indicated the following species of pests associated with *B. carinata*: *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae), *Plutela xylostella* larvae*, Pieris rapae* L. (Lepidoptera: Pieridae), *Diabrotica undecimpunctata* Barber (Coleoptera: Chrysomelidae), *Lipaphis pseudobrassicae* Davis (Hemiptera: Aphididae), *Leptoglossus phyllopus* L. (Hemiptera: Coreidae), and *Chloridea virescens* F. (Lepidoptera: Noctuidae). The insect distribution within the plant canopy was not uniform. Different levels of artificial *B. carinata* defoliation did not affect seed weight, the number of seeds per pod, or the oil content of the seeds. The number of pods per plants and estimated yield were negatively impacted by defoliation during the vegetative and flowering stages.

Keywords: carinata, defoliation, pests, insects, yield impact, defoliation impact estimation from regression curves, one-time defoliation event and continuously defoliation impact estimation.

Introduction

The Energy Independence and Security Act was passed to increase the domestic production of renewable fuels (Energy Independence and Security Act 2007). By 2022, the Renewable Fuel Standard aims to have 136 billion liters of renewable fuel, resulting in a reduction in greenhouse gasses when compared to a 2005 petroleum baseline (Energy Independence and Security Act 2007; EPA 2016; 2017). In this scenario, a link between agriculture and energy sectors has been established with the inclusion of crops providing renewable fuel (Zhengfei and Oh 2015).

Brassica carinata (A. Braun) (Brassicales: Brassicaceae) is grown to produce biofuel, lubricants, bioplastics, and animal feedstock (Taylor et al. 2010). This crop is considered sustainable for biofuel production due to the high erucic acid content of the seed oil (Seepaul et al. 2016), which makes the conversion to biofuel more efficient than traditional oils, such as soybean and corn oils (Cardone et al. 2003). *Brassica carinata* has high yield and oil content compared with other brassicas (Taylor et al. 2010). In addition, large seed size and shattering resistance during maturity and harvest operations are advantages of this crop for biofuel production (Wright et al. 1995). Given the current constraints of high production costs and limited alternative aviation biofuels (Gegg et al. 2014), the potential to use carinata for jet fuel production has been evaluated (SPARC 2017a) as a high energy biofuel crop.

Brassica carinata is commonly known as Ethiopian mustard or Abyssinian mustard and originated in Ethiopia (Kassa 2002). Reports indicate the use of the leaves as food when boiled, and the use of processed seeds to alleviate stomach upset (Kassa 2002). This plant 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). Currently, *B. carinata* is cultivated in Canada, the U.S, and Uruguay (Seepaul et al. 2019). It is more tolerant to heat and drought than canola (*B. napus*), and is well-adapted to cooler climates, although it is vulnerable to freeze damage. Mortality due to freeze damage is especially critical when the root system is shallow, i.e., when it is in the seedling stage, but frost damage at the bolting stage is more likely to result in recoverable damage (Mulvaney et al. 2018). The possible benefits of growing this crop following summer row crops

include reduced leaching, soil erosion, decreased weed seed bank, and improvement of organic matter (Seepaul et al. 2016).

Currently, "Avanza64" is the only commercial cultivar of *B. carinata* available in the Southeast U.S. However, germplasm and elite cultivars are being evaluated to promote *B. carinata* as a winter crop (SPARC 2017a, SPARC 2017b, SPARC 2017c). Research has focused on agronomic management practices, including seeding rate, nutrient management, rotation effects, and harvest aids to incorporate *B. carinata* into the region as a double-crop system (Mulvaney et al. 2019).

As *B. carinata* become more widely cultivated, in the Southeast U.S pest problems will emerge, and an IPM program is needed. *Brassica carinata* is reported to be resistant to many insects (Malik 1990; Getinet et al. 1996). One of the explanations for this resistance is the presence of secondary compounds that provide a chemical defense against herbivores (Halkier and Gershenzon 2006; Speight et al. 2008; Klowden 2013). Members of Brassicaceae are known to synthesize 30 to 40 different glucosinolates from amino acids and glucose (Halkier and Gershenzon 2006). However, some specialist insect herbivores such as *Phyllotreta* spp. (Coleoptera: Chrysomelidae) and *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Thorsteinson 1953; Lamb 1984; Loon et al. 2002; Halkier and Gershenzon 2006) have evolved to feed on *Brassica* spp. and are stimulated to feed by the presence of glucosinolates. Insect pests that cause defoliation in brassicas can have a varying impact on crop yield (Pandey 1983; Rajewski et al. 1991; Ramachandran et al. 2000).

Based on the foundation of IPM, the costs and integration of preventive and curative practices to decrease the economic impact of pests associated with *B. carinata* should be evaluated and validated. However, initial steps are necessary when designing an IPM program for a novel crop, such as *B. carinata* in the Southeast U.S. The objectives of this study were to document the occurrence of arthropod pest species associated with *B. carinata* during different phenological stages, the possible source of host plant resistance by non-preference in the *B. carinata* germplasm, and the impact of defoliation on seed yield. These will serve as the first steps for the development of IPM tactics.

Materials and methods

Pest Survey

The survey of pests associated with *B. carinata* in the Florida Panhandle was performed during two years 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 *B. carinata* in the Southeast U.S. (Seepaul et al. 2016). Planting dates were as follows: the 2017/2018 experiment was planted on November 16, 2017 and the 2018/2019 experiment was planted on December 19, 2018. Sixteen *B. carinata* genotypes were evaluated in the 2017/2018 crop season, coded AX17001 to AX17016. The genotype AX17016 is the current commercial cultivar "Avanza64". The same *B. carinata* genotypes were cultivated during the 2018/2019 crop season, except AX17015. The study was performed using a randomized complete block design with four replications. Each replication was 73.2 m by 9.1 m with 0.381 m row spacing, and fifteen rows per plot. The first and the fifteenth rows were the borders of each 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 arthropod pest species present, number of pests, and the position of the pest on the plant canopy. 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. Specimens of insects detected were identified and recorded. Aphid samples were submitted to the Florida Department of Agriculture and Consumer Services-Division of Plant Industry (FDACS- DPI, protocol E2019-579-1) for species-level identification. Six pest samplings were performed during the 2017/2018 crop season, and pest sampling was not performed during the vegetative stage due to the low temperature during this plant stage. Nine pest samplings were performed during the 2018/2019 crop season.

Defoliation Study

The impact of defoliation on *B. carinata* was determined 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 described above and was arranged as a split-plot randomized complete block 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 *B. carinata* in the Southeast United States (Seepaul et al. 2016). Planting of the 2017/2018 crop season was on February 22, 2018 and harvest occurred on June 28, 2018. Each replication was 38.1 m by 10.7 m. Row spacing was 0.381 m, 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 m by 9.1 m with 0.381 m row spacing, and fifteen rows per plot. The first and the fifteenth rows were the borders of each plot. One application of the pyrethroid Mustang Maxx FMC © (0.06 L/acre) was performed in the experimental area in the initial vegetative stage, in each crop season to avoid natural infestation of defoliating 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 flower buds formed), pod development stage of *B. carinata* was over 50% of plants in the experimental area have pods formed and pod development (50% of plants had mature size pods) (Seepaul, R, *personal communication*).

During the 2017/2018 crop season, five levels (0%, 5%, 25%, 50%, and 100%) of one-time artificial defoliation was performed during each of the three growth stages. During the 2018/2019 crop season, based on the analysis of the data of the 2017/2018 crop season, the levels of defoliation were adjusted to 0%, 50%, 75%, 90%, and 100%. Also, in2018/2019, a continuous defoliation were added. A 50% and 100% continuous defoliation treatments was initiated at the vegetative, flowering, and pod development 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 in each row had at least one non-treated plant between them. In this way, the crop environmental conditions in the plant canopy was kept close to the natural conditions, whitout excessive exposition to sum and wind. 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 different colors of flagging tape to identify their defoliation level.

When plants reached full development, five plants were harvested during the 2017/2018 and 15 plants during the 2018/2019 crop season. The number of pods per plant and the average number of seeds per pod for five plants were counted. The number of seeds per pod was determined by sampling three pods per plant and counting the number of seeds per pod. In the 2018/2019 crop season, an additional 10 plants were used for estimating the seed oil concentration (%), erucic acid (% C22:1) and 1000 seed weight, which is a commonly used measurement to determine seed yield for agronomic crops. Thousand seed weight was determined by weighing 100 seeds from each defoliation level and multiplying the weight by 10. The moisture content was estimated with a plant moisture tester (GEHAKA AGRI, Moisture Tester G600, Sao Paulo, Brazil), and the seed weight was adjusted to 8% moisture content. Seed samples of 15 g from each plot were submitted to Agrisoma Biosciences, Inc (currently NuSeed) (Gatineau, Quebec, Canada) and the oil concentration, and fatty acid composition were estimated (Rathke et al., 2006) using near-infrared reflectance spectroscopy (FOSS XDS Rapid Content Analyzer, FOSS Inc. Delaware, U.S.). The yield was estimated by the following calculation (Sieverding et al., 2016):

$$\text{yield} = \frac{((1000 \text{ seed weight x pods/plant x seeds per pod})/\ 1000) \text{ x plant stand}}{1000}$$

Data Analysis

Differences in pest occurrence and abundance among *B. carinata* genotypes and plant stages were analyzed using ANOVA (R Core Team, 2018). Genotype and plant stage were fixed variables,

replication was the random factor, and the number of pests was the response variable. The data collected during the 2017/2018 and 2018/2019 crop seasons were treated and analyzed as separate experiments. 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 multiplying by 100. Pest canopy position and number of pests were graphed as balloon plots (R Core Team 2018). Data was not transformed, and the differences were tested at a 95% confidence interval.

An ANOVA was performed to test for differences in the number of the pods per plant, number of seeds per pod and crop yield at 8% moisture under different defoliation levels at different *B. carinata* phenological stages. 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, the number of seeds per pod, and yield (Pedigo et al., 1986, Paula-Moraes et al., 2013). The data analysis program used was "R" (R Core Team 2018). A p-value was evaluated at the 95% confidence interval.

Results

Pest Survey

During the 2017/2018 crop season, the occurrence of pests associated with *B. carinata* was not significantly different across crop stages ($F = 0.119$; $df = 2$; p-value = 0.73). Similarly, the occurrence of pests among different genotypes of *B. carinata* was not significantly different ($F = 0.846$; $df = 2$; p-value = 0.358). The pest species detected in *B. carinata* during the 2017/2018 crop season included *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae) 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 1). In Figures 1 and 2, the size of the balloons is proportional to the number of insects

of each species associated with *B. carinata.* Most of the pests observed on *B. carinata* were located on the lower canopy (Figures 1 and 2).

During the 2018/2019 crop season, the occurrence of pests was significantly different across crop stages of *B. carinata* ($F = 10.6$; $df = 2$; p-value = 1.60×10^{-4}), but the occurrence of pests was not different across the 15 genotypes ($F = 0.077$; $df = 1$; p-value = 0.781). 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, *D. undecimpunctata*, and *Chloridea virescens* F. (Lepidoptera: Noctuidae) larvae (Table 1). During the vegetative stage in the 2018/2019 crop season most pests were in the upper plant canopy. During flowering stage, most pests were in middle plant canopy (Figure 4), and during the pod development stage, most pests were located in the lower plant canopy (Figure 5).

Adults and larvae of *L. pseudobrassicae, M. ochroloma* adults and larvae and *P. xylostella* larvae did not have a uniform plant canopy distribution (Figures 1- 5). *Pieris rapae* larvae, *L. phyllopus* adults, *D. undecimpunctata*, and *C. virescens* larvae were observed at low densities, and no differences in plant canopy distribution were observed (Table 1; Figures 1 - 5).

Defoliation Study

During the 2017/2018 crop season, the number of seeds per pod did not differ in plants submitted to one-time defoliation during the vegetative ($F = 1.483$; $df = 4$; p-value = 0.216), flowering ($F = 4.582$; $df = 4$; p-value = 0.078), and pod development stages ($F = 2.83$; $df = 1$; p-value = 0.094). Similarly, during the 2018/2019 crop season, the number of seeds per pod was not different in plants subjected to one-time defoliation at the vegetative ($F = 1.21$; $df = 4$; p-value = 0.313), flowering ($F = 0.816$; $df = 4$; p-value = 0.518), and pod development stages ($F = 2.83$; $df = 1$; p-value = 0.096). In the 2018/2019 crop season the 1000 seed weight was not different among defoliation levels at vegetative ($F = 1.92$; $df = 8$; p-value = 0.116), flowering ($F = 0.301$; $df = 7$; p-value = 0.945), and pod development ($F = 0.847$; $df = 6$; p-value = 0.548) stages.

The oil concentration of seeds were also determined for the 2018/2019 crop season and was not significantly different among plants that were submitted to one-time defoliation at the vegetative

(F = 2.66; df = 1; p-value = 0.120), flowering (F = 1.489; df = 4; p-value = 0.258), and pod development stages (F = 0.504; df = 4; p-value = 0.487). The average seed oil concentrations were as follows: 44.15% (SD ± 1.74), 45.77% (SD ± 1.66), and 45.31% (SD ± 2.06), when one-time defoliation were performed during the vegetative, flowering, and pod development (Table 2). Seed oil erucic acid concentration was also not different between levels of defoliation at the vegetative (F = 0.0.22; df = 1; p-value = 0.644), flowering (F = 0.0.034; df = 1; p-value = 0.856), and pod development stages (F = 0.416; df = 1; p-value = 0.527) (Table 2).

The linear relationship between defoliation and number of pods per plant when one-time defoliation was performed at the vegetative stage 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). The reduction in number of pods per defoliation unit (1% defoliation) occurred at a rate of 0.86 and 0.96, respectively (Table 3). When one-time defoliation was performed at flowering, the linear relationship between defoliation and the number of pods per plants 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). The reduction in the number of pods per defoliation unit in 2017/2018 and 2018/2019 occurred at a rate of 0.54 and 0.58 per percentage unit of defoliation, respectively. At the pod development 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).

The mean number of the pods per plant from plants that were submitted to defoliation levels at the vegetative stage were separated by Tukey's test, and indicated a significant difference in the number of pods per plant, in both the 2017/2018 (F = 7.49; df = 4; p-value = 8.76×10^{-5}) and the 2018/2019 (F = 11.59; df = 4; p-value = 1.9×10^{-7}) crop seasons. Similarly, the number of the pods per plant were impacted when plants were defoliated at the flowering stage in both the 2017/2018 (F = 4.58; df = 4; p-value = 2.3×10^{-4}) and the 2018/2019 (F = 5.67; df = 4; p-value = 3.9×10^{-5}) crop seasons. The negative impact of defoliation on the number of the pods per plant was consistently observed above 50% defoliation at vegetative and flowering stages, in both 2017/2018 and 2018/2019 crop seasons (Figures 6 and 7). Defoliation at pod formation stage did not impact the number of the pods per plant in both crop seasons, (Figures 6 and 7).

Brassica carinata yield was estimated in kg/ha during the 2018/2019 crop season (Table 4). The yield was reduced when the plants were one-time defoliated at vegetative (p-value = 2.2×10^{-5}, $R^2 = 0.51$) and flowering (p-value = 0.0169, $R^2 = 0.23$) stages (Table 4). The yield reduction was 21.69 and 8.23 kg/ha respectively per 1% defoliation (Table 4). Defoliation did not impact yield when plants were submitted to one-time defoliation at the pod development stage (p-value = 0.933, $R^2 = -0.05$).

The continuous defoliation study, starting defoliation at vegetative or at flowering stages during the 2018/2019 crop season, presented similar results to the one-time defoliation. the relationship between, The number of pods per plant 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 per plant per percentage of defoliation (Table 3).

Continuous defoliation starting at vegetative and flowering stages also impacted yield. The yield response to continuous 50% and 100% defoliation beginning at the vegetative (p-value = 1.24×10^{-6}, $R^2 = 0.37$) and flowering (p-value = 0.0021, $R^2 = 0.59$) stages had a linear relationship (Table 4). However, the yield of *B. carinata* was not impacted when the plants were submitted to continuous defoliation during the pod development stage (p-value = 0.8413, $R^2 = -0.09$) (Table 4). The yield reduction in plants submitted to defoliated during the vegetative and flowering stages was 30.84 and 13.35 kg/ha per unit of defoliation, respectively (Table 4).

Discussion

The present study was conducted during two winter/spring crop seasons in the Florida Panhandle and represents the first steps for the development of IPM tactics for this novel crop. Results indicated the presence of *M. ochroloma*, *P. xylostella, P. rapae*, *D. undecimpunctata*, *L. pseudobrassicae*, *L. phyllopus,* and *C. virescens.* The population density of each species was variable, but these findings indicate that there are several insects species that utilize *B. carinata* as a host in the southeast United States. Several pests have been associated with species of *Brassica* spp. in the southeast U.S. (Ramachandran et al. 2000; Loon et al. 2002; Reddy 2017; Manrique et al. 2012); however, this is the first report of pests associated with *B. carinata*. *Plutella xylostella* is a pest

associated with brassica causing annual yield losses and management costs estimated at $2.3 billion (Zalucki et al. 2012). Besides brassicas, *P. xylostella* has 20 wild and cultivated plant hosts, including members of the families Malvaceae, Fabaceae, and Asteraceae (CABI 2018). During both seasons, this species was associated with *B. carinata*, and during the 2018/2019 crop season, this species occurred in all crop stages. Aphid species are also 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 southeastern U.S. are *Lipaphis erysimi* Kaltenbach*, Brevicoryne brassicae* (L.), and *Myzus persicae* Sulzer (Reddy 2017). Mezgebe et al. (2018) reported *B. carinata* as a suitable host for *B. brassicae.* However, the aphid species associated with *B. carinata* during both crop seasons was *L. pseudobrassicaei,* which was also the most abundant insect associated with *B. carinata*. Future studies should include an investigation of the effects of aphids in this crop.

Microtheca ochroloma is an economic pest of *Brassica* species in the southeastern U.S. (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), butit has other *Brassica* species as host plants (Ameen 1996; Balusu and Fadamiro 2011). Multiple and overlapping generations of *M. ochroloma* can occur during the winter/spring crop season (Reddy 2017).

The least common pests detected in both crop seasons were *P. rapae, D. undecimpunctata*, *L. phyllopus*, and *C. virescens*. In the U.S., *P. rapae* has been documented as a minor pest in canola and there are no reports documenting this species as an economic pest (Bucur and Rosca 2011). Based on the present study, *P. rapae* was not abundant in *B. carinata* and is expected to be at most a minor pest of *B. carinata* in the southeastern United States. (Ma et al. 2009; (Jackson et al. 2005). However, *Diabrotica* species have a large host range, including several *Brassica* species such as canola, *B. oleracea* var. capitata, and *B. rapa* subsp. *chinesis* (Walsh 2003). *Diabrotica undecimpunctata* was only found in the adult stage and in low numbers on *B. carinata* during the *B. carinata* reproductive stage. Future pest surveys of *B. carinata* should include root sampling to determine if *B. carinata* is a host for this pest during the larval stage. *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, during the 2018/2019 crop season, *L. phyllopus* was found in low abundance feeding on *B. carinata* during the pod development stage. *Chloridea virescens* was found once during *B. carinata* pod development during the 2018/2019 crop season. This is a polyphagous pest of field crops and has been reported feeding on *Brassica* species (Capinera 2012). The broad adoption of transgenic cotton expressing insecticidal toxins of the bacteria *Bacillus thuringiensis* has been suppressing populations of this pest in the agricultural landscape of the Southeast U.S. (Abney et al. 2007).

Differences in pest abundance during the two crop seasons were observed. The 33-day difference in planting dates between the two seasons (2017/2018: November 16th, 2017 and 2018/2019: December 19th, 2018) resulted in temperature differences during crop growth and development, which could have influenced the pest abundance. These differences in temperature are representative of the variation in the abiotic factors during the winter/spring crop season in the southeast U.S, which influence the annual occurrence and abundance of pests in the region. The documentation of species of insects associated with *B. carinata* in the present study indicates its potential 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 southeast U.S., considering the establishment of *B carinata* as a winter/spring crop for the region. Pest species that may be associated with *B. carinata* are expected to have multiple generations (Reddy 2017). In cases where a summer crop is planted just after harvest of *B. carinata* and the summer crop is a suitable host plant of the insect species previously listed, the summer crop could have a higher probability of economically damaging levels of pest infestation than if the land had been fallow (Altieri et al. 1984). On the other hand, the populations of natural enemies that could build 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 early in the summer crop season.

The pest distribution in the *B. carinata* canopy was evaluated since it is a critical aspect of the development of varios components of in a *B. carinata* IPM program. The pest canopy distribution can be influenced by a variety of factors, including preference for pest feeding sites (Paula-Moraes et al. 2012) and pest behavior (Pencoe and Lynch 1982; Paula-Moraes et al. 2012). Some species of

Lepidoptera prefer certain oviposition zones within a canopy. For example, *Mamestra configurata* (Walker) prefers the upper canopy of *Brassica* spp. (Ulmer 2002). *Plutella xylostella* neonates prefer feeding on the youngest leaves of cabbage, so oviposition occurs more frequently on younger leaves than older leaves (Ang et al. 2014). *Plutella xylostella* adults prefer waxy leaves for oviposition sites (Ulmer 2002; Musser et al. 2005). In *Brassicas* spp. the presence of secondary compounds within the leaves may also affect the pest distribution in the canopy. Glucosinolates have been reported in both the leaf and stem tissues of *Brassica* spp., but the composition of glucosinolates can vary among leaves at different ages and positions (Porter et al. 1991) and can also change throughout the plant life cycle (Bellostas et al. 2004; Gols et al. 2018). Sampling during two crop seasons indicated that *P. xylostella* larvae were detected in all canopy zones during the vegetative, flowering, and pod development stages. Similarly, larvae and adults of *M. ochroloma* were observed in all canopy zones. In the case of *L. pseudobrassicae,* no canopy distribution pattern was detected. According to Reddy (2017), canopy distribution of *L. pseudobrassicae* would be predominant in 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 during both vegetative and flowering stages. However, during the 2017/2018 crop season, *L. pseudobrassicae* were most prevalent in the lower canopy. This lower canopy distribution followed the same distribution pattern reported by Sampaio et al. (2017) for *L. pseudobrassicae* when feeding on *B. oleracea.* In this study, the authors the tested the effect of parasitoids, precipitation and temperature effects and none of these factors indicatd to be determinants for the canopy position of *L. pseudobrassicae.*

Host plant resistance is a low-input management strategy that should be explored in an IPM program for *B. carinata*, and the genotypes evaluated here are currently under evaluation for the development of commercial cultivars. The documentation of pests associated with *B. carinata* was performed in 16 genotypes of *B. carinata.* However, there were no differences in the occurrence of pests listed in the present study across the 16 genotypes, and consequently, any source of plant resistance was not identified among the genotypes evaluated.

The results of the pest survey indicated that many of the pests associated with *B. carinata* are defoliators. Different levels of artificial *B. carinata* defoliation did not affect seeds weight, the number of seeds per pod, or the oil content of the seeds. Major et al., (1978) indicate that the lower

leaves of canola contribute photosynthates that are sent to the roots, while the upper leaves and stems contribute photosynthates to the pods and seeds. The authors also reported that pods photosynthesize but do not transport assimilates outside of the pod, although they do serve as a sink for assimilates from the upper leaves and stems. King et al. (1997) suggested that the pod wall contributes carbohydrates to seed development. These results agree with the previous report of the impact of defoliation in canola (Ramachandran et al. 2000), which indicated that the impact of defoliation on seed production depends on the crop stage. Canola is also an oil seed plant and the tissues allocate resources to different portions of the plant (Major et al., 1978).

Different crops have different yield responses in the oil content when submitted to defoliation. Soybean (*Glycine max* (L.) Merr.) does not have a decrease in seed oil content when submitted to defoliation (Proulx and Naeve 2009). However, removal of soybean nodes and consequently the foliar parts attached to the nodes on main stems at 80% or higher during the early crop stages can decrease seed oil content (Conley et al. 2009). Canola does not have a decrease in seed oil content until it is defoliated at 100% (Ramachandran et al. 2000; Proulx and Naeve 2009), and this is similar to our findings with *B. carinata*. The contributions of the upper stems and pod wall likely aid the plant in maintaining seed oil content (Major et al., 1978; King et al. 1997).

Our findings indicate that *B. carinata* is tolerant of low levels of defoliation (<50%) during the vegetative and flowering stages. Yield was impacted when defoliation was over 50% at the vegetative and flowering stages. Defoliation above 50% might have caused the plant to allocate more resources into the vegetative tissues, similar to what has been reported in canola (Ramachandran et al., 2000; Mccormick et al. 2013). The reduction in the number of the pods per plant and consequently, reduction in the number of seeds per plants result in the decrease of the amount of oil yield, which is the primary marketable component of *B. carinata* (Seepaul et al. 2019). Defoliation at the pod development stage did not impact the number of pods per plant or seeds per plant. This could be because, during this late stage, the number of the pods per plant has been set, and no abortion of reproductive parts were occurred.

Based on Pedigo et al. (1986), the pest damage regression curve was estimate for the first time for *B. carinata*. Linear regression analyses were performed including all levels of defoliation imposed to *B. carinata* in each crop stage. The significance of the linear curves was tested and the negative

slope of the curve was used as a estimator of the maximum yield loss (kg/ha) of this crop per percentage of plant defoliation. The linear regression equations for the response of seed yield to defoliation levels during the 2018/2019 crop season indicated that defoliation during vegetative and flowering stages had a yield reduction of 21.69 ($R^2 = 0.51$) and 8.23 kg/ha ($R^2 = 0.23$) per percentage of defoliation, beyond 50%, respectively. The results of seed yield impact in plants submitted to continuous defoliation during vegetative and flowering stages agree with the results previously presented. The yield reduction was 30.84 ($R^2 = 0.37$) and 13.45 kg/ha ($R^2 = 0.59$) per percentage of defoliation, beyond 50%, respectively.

Economic injury level (EIL) is one of the major components of an IPM program. The rate of yield loss per percentage of defoliation when plants for *B. carinata* were submitted to defoliation at the vegetative and flowering stages were estimated. This is the first step for the development of EIL for pests that cause defoliation in *B. carinata.* Moreover, the maximum rate of yield loss per percentage of defoliation (Pedigo et al. 1986) was also estimated for plants submitted to continuous defoliation. The yield reduction in continuously defoliated plants at vegetative and reproductive stages were approximately 30 and 38% more impacted, respectively, compared with *B. carinata* yield of plants submitted to a one-time defoliation event. Future studies for the development of EILs for *B. carinata* defoliation should be performed to document the equivalent impact that specific defoliator pests, such as *P. xylostella* and *M. ochroloma* can cause to *B. carinata*. Future defoliation impact studies should be estimated considering specific pest feeding patterns, resulting consumed foliar area , and canopy reduction (e.g. leaf area index reduction). The yield impacts resulting from scenarios of continuous defoliation should be selected for these comparisons and development of EILs, since they represent more realistic scenarios of pest injury, especially in regions with high pest pressure and multiple generations, as is expected in the southeast U.S.

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Data Sharing and Accessibility

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Tables

Table 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 at 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 multiplying by 100.

Vegetative stage of *B. carinata* was defined as over 50% of plants with no open flowers or developed pods.

Flowering stage of *B. carinata* was defined as over 50% of plants in the experimental area with flower buds formed. Pod development stage of *B. carinata* was over 50% of plants in experimental area had pods formed.

Table 2. Average oil and erucic acid concentrations in *B. carinata* seeds submitted to one-time defoliation at different phenological stages during the 2018/2019 crop season at Jay, FL.

Crop stage	Average seed oil concentration (%)	SD	Average erucic acid concentration (% C22:1)	SD
Vegetative	44.15	1.74	43.60	1.43
Flowering	45.77	1.66	45.76	1.28
Pod development	45.31	2.06	43.15	1.21

Table 3. Linear regression equations describing pods per plant of *Brassica carinata* vs. defoliation percentage at different crop phenological stages, during the 2017/2018 and 2018/2019 crop seasons at 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 – one time defoliation				
Vegetative	$\hat{y} = 104.49 - 0.86x$	0.143	8.01	3.19×10^{-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 – one time defoliation				
Vegetative	$\hat{y} = 150.29 - 0.96x$	0.14	10.39	1.03×10^{-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	3.12×10^{-7}*	0.37
Flowering	$\hat{y} = 147.15 - 0.71x$	0.11	7.39	5.23×10^{-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 were 0%, 5%, 25%, 50%, and 100 %. 2018/2019 levels of defoliation were 0%, 50%, 75%, 90%, 100%. Continuous defoliation during 2018/2019 were 50% and 100% applied during the specific crop phenological stage.

* Indicates significance at $p<0.05$

"n.s." indicates not significance at $p\geq0.05$

$\hat{y}$ = pods/plant

a = intercept (kg/ha)

b = slope (kg/ha)

x = defoliation %

Table 4. Linear regression equations for the relationship between estimate yield (kg/ha) of *Brassica carinata* and defoliation levels, by crop phenological stage during the 2018/2019 crop season. 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**
	2018/2019 – one time defoliation				
Vegetative	$\hat{y}$ = 3,210.34 - 21.69x	341.65	4.72	2.2×10^{-5}*	0.51
Flowering	$\hat{y}$ = 2,539.66 - 8.23x	226.32	3.12	0.0169*	0.23
Pod development	$\hat{y}$ = 1,765.93 – 6.39x	370.87	5.12	0.933 n.s.	-0.05
	2018/2019 - Continuous defoliation				
Vegetative	$\hat{y}$ = 3,750.93 – 30.84x	193.67	3.01	1.24×10^{-6}*	0.37
Flowering	$\hat{y}$ = 3,316.44 - 13.45x	210.78	3.26	0.0021*	0.59
Pod development	$\hat{y}$ = 2,893.89 - 1.67x	525.84	8.14	0.8413 n.s.	-0.09

2018/2019 levels of defoliation were 0%, 50%, 75%, 90%, 100%, and 100% continuous defoliation.

* Indicates significance at $p<0.05$

"n.s." indicates not significant ($p \geq 0.05$)

$\hat{y}$ = yield (kg/ha)

a = intercept (kg/ha)

b = slope (kg/ha)

x = defoliation %

Figures

Figure 1. Distribution and frequency of insect species in different *Brassica carinata* canopy zones at the flowering stage during the 2017/2018 winter/spring crop season at Jay, FL.
The numbers with the figure represent the total number of insects within the sampling period.
The p-value comes from the chi-square analysis comparing each species against itself by canopy position.
The size of the balloon is proportional to the number of insects of each species associated with *B. carinata.* The shading on the upper x-axis shows what proportion of the insects that were found in that plant canopy zone. The gray highlight on the y-axis shows the proportion of the insects per each species.

Figure 2. Distribution and frequency of insect species in different *Brassica carinata* canopy zones at the pod development stage during the 2017/2018 winter/spring crop season at Jay, FL.
The number labels on the figure represent the total number of insects within the sampling period.
The p-value comes from the chi-square analysis comparing each species against itself by canopy position.
The size of the balloons is proportional to the number of insects of each species associated with *B. carinata.* The shading on the upper x-axis shows what proportion of the insects that were found in that plant canopy zone. The gray highlight on the y-axis shows the proportion of the insects per each species.

Figure 3. Distribution and frequency of insect species in different *Brassica carinata* canopy zones during the vegetative stage during the 2018/2019 winter/spring crop season. Jay, FL.
The number labels on the figure represent the total number of insects within the sampling period.
The p-value comes from the chi-square analysis comparing each species against itself by canopy position.
The size of the balloons is proportional to the number of insects of each species associated with *B. carinata.* The shading on the upper x-axis shows what proportion of the insects that were found in that

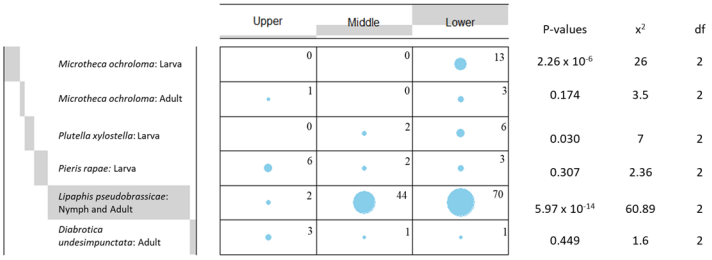
plant canopy zone. The gray highlight on the y-axis shows the proportion of the insects per each species.

Figure 4. Distribution and frequency of insect species in different *Brassica carinata* canopy zones during the flowering stage during the 2018/2019 winter/spring crop season. Jay, FL.
The number labels on the figure represent the total number of insects within the sampling period.
The p-value comes from the chi-square analysis comparing each species against itself by canopy position.
The size of the balloons is proportional to the number of insects of each species associated with *B. carinata.* The shading on the upper x-axis shows what proportion of the insects that were found in that plant canopy zone. The gray highlight on the y-axis shows the proportion of the insects per each species.

Figure 5. Distribution and frequency of insect species in different *Brassica carinata* canopy zones at the pod development stage during 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 size of the balloons is proportional to the number of insects of each species associated with *B. carinata.* The shading on the upper x-axis shows what proportion of the insects that were found in that plant canopy zone. The gray highlight on the y-axis shows the proportion of the insects per each species.

Figure 6. Number of pods per plant of *Brassica carinata* under different one-time defoliation levels during different crop growth stage during the 2017/2018 crop season at Jay, FL. Means with the same letter within each crop phenological stage were not significantly different (Tukey, p-value ≤ 0.05). Defoliation levels were imposed within their crop stage.

Figure 7. *Brassica carinata* pods per plant under different one-time defoliation levels at different crop phenological stages during the 2018/2019 season at Jay, FL. Means with the same letter in each crop phenological stage were not significantly different (Tukey, $p \leq 0.05$). Defoliation levels were tested within their crop stage.



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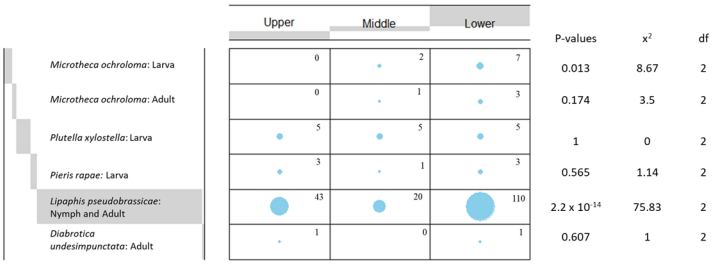

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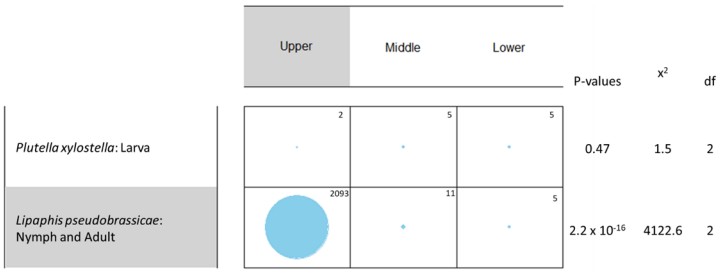

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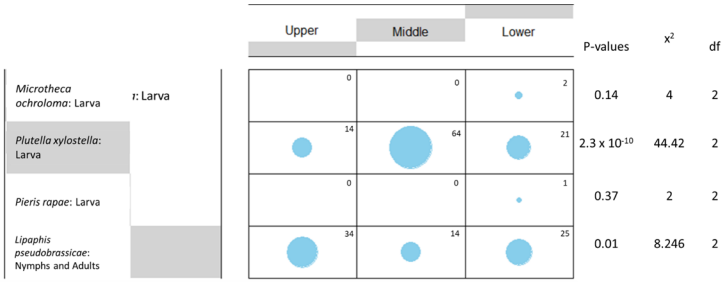

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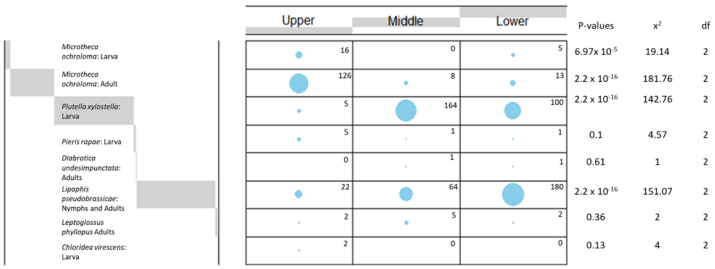

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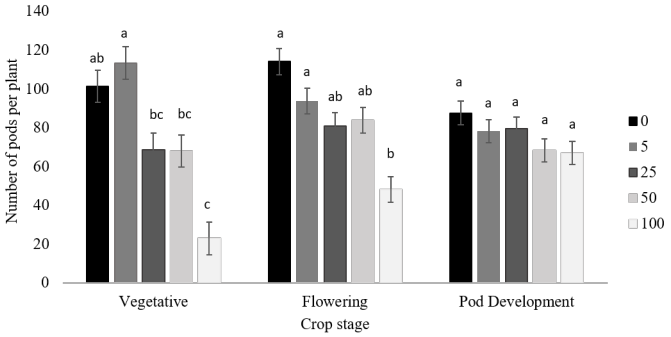

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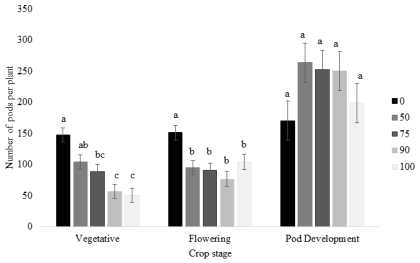

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