Resistance to fall armyworm (Lepidoptera: Noctuidae) feeding identified in nascent allotetraploids cross-compatible to cultivated peanut (*Arachis hypogaea* L.)

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ABSTRACT

Fall armyworm (FAW) is an economically devastating, invasive pest in Sub-Saharan Africa and can be a major pest in the Americas. This pest feeds on more than 80 plant species, including peanut, and threatens the food security of millions of people who rely on these crops in Sub-Saharan Africa. An integrated pest management strategy, including resistant crop cultivars, is needed to control FAW, since populations have been reported to develop insecticide resistance. Genetic sources of host resistance to FAW are limited in cultivated peanut; however, strong resistance to FAW was reported previously in peanut wild relatives. In this *in vitro* study, we tested diploid peanut relatives including *A. ipaensis* KG37006 (*Ipa*), *A. duranensis* 30060 (*Dur*), *A. correntina* 9530 (*Cor*9530), and *A. correntina* 9548 (*Cor*9548); allotetraploids including *IpaCor*9530^{4x}, *IpaDur*^{4x}; F_2 hybrids [*A. hypogaea* 13-1014 x *IpaCor*9530^{4x}]; and cultivated peanut lines *A. hypogaea* '13-1014′ and 'Georgia Green' for FAW resistance to identify valuable materials in our breeding program. FAW development was measured by survival, larval weight, larval stage duration, pupation, pupal stage duration, moth emergence relative to pupation, and moth sex. All allotetraploids showed promise as donors for FAW resistance. This FAW resistance was derived primarily from *A. ipaensis*, but *A. duranensis* was also identified as a source of resistance, though more moderate. A high level of heterogeneity was found in *A. correntina* 9530, which likely contributed to the variable performance of this species in the bioassay. Producing hybrids and allotetraploids with wild *Arachis* species does not guarantee that each derived line from these crosses will be resistant, and since these lines are segregating, selection for resistance is still needed.

Key Words: Antibiosis, *Arachis*, host plant resistance, *Spodoptera frugiperda*, *in vitro*.

Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a major defoliating pest in the Americas and has recently become an economically devastating, invasive pest in Sub-Saharan Africa (Sparks, 1986; Goergen *et al*., 2016), as well as in Asia. FAW feeds on more than 80 economically important crops, including peanut. A thirteen billion dollar loss to FAW infestation was reported for maize, sorghum, rice, and sugarcane in Africa from 2016 to 2017 (Abrahams *et al*., 2017). Without control, FAW is a major defoliator that can cause high yield reductions, seriously affecting production in developing countries with limited access to pesticides and safety equipment to apply them properly (Cock *et al*., 2017; Day *et al*., 2017; Bateman *et al*., 2018; CABI, 2020). Producing FAW resistant cultivars will reduce reliance on pesticides, increase food security, and promote sustainable agriculture in these at-risk countries. In addition, resistant cultivars are needed to mitigate the insecticide resistance reported in FAW populations (Yu, 1991).

Genetic sources with strong FAW resistance are limited in cultivated peanut due to its narrow genetic base (Stalker *et al*., 2016). Peanut is an allotetraploid species (AABB; $2n$=$4x$=40), and evidence suggests that it arose from a natural polyploidization event involving hybridization of the diploid species *A. ipaensis* (BB; $2n$=$2x$=20) and *A. duranensis* (AA; $2n$=$2x$=20) (Bertioli *et al*., 2016). The difference in ploidy levels between cultivated peanut and its wild relatives results in crossing barriers and constricts genetic diversity in peanut. Moderate levels of host resistance to FAW have been found in a few peanut cultivars such as 'Southeastern Runner 56-15′ (Hammons 1970, Leuck and Skinner 1971), 'Florunner' and 'Tifton 8′ (Todd *et al*., 1991). On the other hand, more than 80 wild peanut relatives have diverse levels of resistance against a wide range of peanut insect pests and diseases (Stalker *et al*., 2016; Stalker,

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2017). Wild peanut relatives, such as *Arachis cardenasii*, already have proved to be useful sources of resistance to multiple pests including late leaf spot, early leaf spot, root-knot nematode, Cylindrocladium black rot, and Sclerotinia blight (Simpson and Starr, 2001; Stalker and Mozingo, 2001; Gowda *et al*., 2002; Simpson *et al*., 2003; Tallury *et al*., 2014). Previous studies have identified wild peanut relatives such as *A. cardenasii*, *A. correntina*, and *A. villosa* as potential donors of strong FAW antibiosis and antixenosis (Painter, 1951; Kogan and Ortman, 1978; Lynch *et al*., 1981; Ortega *et al*., 2016). These *Arachis* species displayed antibiosis through high FAW mortality rates and inhibition of FAW development and displayed antixenosis due to FAW non-preference to feed on these materials (Lynch *et al*., 1981). In addition, *A. ipaensis* showed antibiosis through a high FAW mortality rate (Yang *et al*., 1993). However, further work has not resulted in FAW resistance being introgressed from these wild relative species into a cultivated background useful for peanut breeders. This study identified FAW resistance in newly created allotetraploids that are cross compatible to cultivated, allotetraploid peanut. Resistant allotetraploid lines identified in this study can be used in breeding programs in the United States and shared with breeding programs in East and West Africa to introgress FAW resistance into elite cultivars. The long-term goal of this study is to create FAW resistant peanut cultivars that can protect yields in the United States and increase yields in regions with limited access to pesticides in Africa.

Materials and Methods

Plant Materials

Wild *Arachis* species, *A. ipaensis* Krapov. and W.C. Gregory (PI 468322, GKBSPSc 30076; abbrev.: *Ipa*), *A. duranensis* Krapov. and W.C. Gregory (PI 468197, GKBSPSc 30060; abbrev.: *Dur*), *A. correntina* (Burkart) Krapov. and W.C. Gregory (PI 262808, GKP 9530; abbrev.: *Cor*9530) and (PI 262881, GKP 9548; abbrev.: *Cor*9548) were grown and the diploid hybrids generated in 2016 at North Carolina State University (NCSU). *IpaDur*^{4x} and *IpaCor*9530^{4x} allotetraploids were created from the diploid hybrids by colchicine treatment of F_1 hybrid cuttings at the University of Georgia (UGA) Tifton Campus. The susceptible *A. hypogaea* controls included 'Georgia Green' (Branch, 1996) and '13-1014,' a breeding line selected from [(C1805-617-2 x 'Florida-07′ (Gorbet and Tillman, 2009)) x 'Georgia-06G' (Branch, 2007)]. C1805-617-2 is a selection from 'Tifguard' (Holbrook *et al*., 2008) x 'Florida-07′.

Due to limitations of labor and pesticide-free plants required for bioassay, the resistance evaluation was performed in three experiments with *Ipa* and allotetraploids with *Ipa* as one parent being tested in all of the experiments (Table 1). The first experiment, aimed to test the level of resistance in *IpaDur*^{4x}, was conducted in July 2018 and included seven genotypes as treatments: *Ipa*, *Dur*, Georgia Green, and four S_2 generation *IpaDur*^{4x} plants. The allotetraploids were each designated an arbitrary number to make distinguishing them easier (Table 1). *IpaDur*_1 and *IpaDur*_2 originated from the same *IpaDur*_$S_{0:1}$ plant, while *IpaDur*_3 and *IpaDur*_ 4 originated from the same *IpaDur*_$S_{0:5}$ plant. The second experiment was designed to test the level of resistance in *IpaCor9530*^{4x}; it was carried out in May 2019 and included 10 genotypes as treatments: *Ipa*, *Cor*9530, *Cor*9548, *A. hypogaea* 13-1014, three S_1 generation *IpaCor9530*^{4x} plants, and three F_1 plants made from a cross between *A. hypogaea* 13-1014 and three different S_0 *IpaCor9530*^{4x} plants (Table 1). *Cor* (PI 261870) was previously reported to have FAW resistance (Lynch *et al*., 1981), but was not included in this study due to lack of seed supply. The third experiment was conducted in November 2019 to directly compare the level of resistance detected in materials from the first two experiments. The third experiment included eight genotypes as treatments: *Ipa*, *Dur*, *Cor*9530, Georgia Green, *A. hypogaea* 13-1014, one *IpaDur*^{4x} S_2 plant, and two *IpaCor9530*^{4x} S_1 plants (Table 1). *IpaDur*_5 was an S_2 plant that originated from one of the *IpaDur*^{4x} lines tested in the July 2018 experiment. *IpaCor*_4 originated from *IpaCor*_1 tested in the May 2019 experiment, while *IpaCor*_5 originated from *IpaCor*_2. In this last experiment, four genotypes were terminated early because the plants grew slowly in the short day length season and failed to produce enough leaves to complete the test. The earliest date of termination was 17 DAI (days after infestation) for genotype *Dur*.

Fall Armyworm Resistance Evaluation

For each of the three experiments, seeds were coated in Vitavax PC (Vitavax, Crompton, Middlebury, CT) and treated overnight in 0.5% Florel Growth Regulator (Lawn and Garden Products Inc., Fresno, CA) to break dormancy. Seeds were then planted in #123 7.62 cm round x 11.43 cm deep Jiffy Pots (Harris Seeds, Rochester, NY) and transplanted approximately one month later into 121.92 cm round x 27.94 cm deep pots filled with Promix growth medium (Premier Tech Horticulture, Quakertown, PA). Normal plant management

Table 1. Genetic materials tested in each fall armyworm feeding bioassay and their abbreviations and ploidy level

July 2018 Bioassay: Plant Materials	Abbreviation	Ploidy Level
Georgia Green	**Georgia Green**[a]	Tetraploid
A. ipaensis 30076	***Ipa***	Diploid
A. duranensis 30060	***Dur***	Diploid
*IpaDur*30060^{4x}_$S_{0:1}$_$S_{1:2}$	*IpaDur*_1	Tetraploid
*IpaDur*30060^{4x}_$S_{0:1}$_$S_{1:4}$	*IpaDur*_2	Tetraploid
*IpaDur*30060^{4x}_$S_{0:5}$_$S_{1:2}$	*IpaDur*_3	Tetraploid
*IpaDur*30060^{4x}_$S_{0:5}$_$S_{1:4}$	*IpaDur*_4	Tetraploid
May 2019 Bioassay: Plant Materials	Abbreviation	Ploidy Level
A. hypogaea 13-1014	**13-1014**	Tetraploid
A. ipaensis 30076	***Ipa***	Diploid
A. correntina 9530	***Cor*9530**	Diploid
A. correntina 9548	*Cor*9548	Diploid
IpaCor9530^{4x}_$S_{0:2}$_S_1	*IpaCor*_1	Tetraploid
IpaCor9530^{4x}_$S_{0:5}$_S_1	*IpaCor*_2	Tetraploid
IpaCor9530^{4x}_$S_{0:6}$_S_1	*IpaCor*_3	Tetraploid
(13-1014 x *IpaCor*9530^{4x}_$S_{0:2}$)_$F_{1:4}$_F_2	(13-1014 x *IpaCor*_1)_F_2	Tetraploid
(13-1014 x *IpaCor*9530^{4x}_$S_{0:5}$)_$F_{1:4}$_F_2	(13-1014 x *IpaCor*_2)_F_2	Tetraploid
(13-2113 x *IpaCor*9530^{4x}_$S_{0:6}$)_$F_{1:4}$_F_2	(13-1014 x *IpaCor*_3)_F_2	Tetraploid
November 2019 Bioassay: Plant Materials	Abbreviation	Ploidy Level
Georgia Green	**Georgia Green**	Tetraploid
A. hypogaea 13-1014	**13-1014**	Tetraploid
A. ipaensis 30076	***Ipa***	Diploid
A. duranensis 30060	***Dur***	Diploid
A. correntina 9530	***Cor9530***	Diploid
*IpaDur*30060^{4x}_$S_{2:1}$	*IpaDur*_5[b]	Tetraploid
*IpaCor*9530^{4x}_$S_{0:2}$_$S_{1:9}$	*IpaCor*_4 [c]	Tetraploid
*IpaCor*9530^{4x}_$S_{0:5}$_$S_{1:7}$	*IpaCor*_5 [c]	Tetraploid

[a]Bolded genotypes were tested in more than one experiment
[b]*IpaDur*_5 originated from one of the *IpaDur* lines tested in the July 2018 experiment
[c]*IpaCor*_4 originated from *IpaCor*_1 and *IpaCor*_5 originated from *IpaCor*_2

was applied in the greenhouse except that insecticide treatments were withheld.

When plants were three months old, FAW eggs (corn strain) were obtained from USDA-ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS. Thirty replications were tested for each genotype, in which one replication comprised one FAW larva fed on the excised leaflets from a genotype. Within 24 h of egg hatch, the neonate larvae were gently transferred with a soft tip paintbrush from the bag of FAW egg masses onto a fresh, newly emerged leaflet placed in a 100 mm x 15 mm Petri dish (ThermoFisher Scientific). Each Petri dish contained a sheet of 9 cm diameter Whatman No. 1 filter paper (ThermoFisher Scientific) supported by a cotton round (Equate Beauty) that was saturated with approximately 4 ml of deionized water. The day that the neonate larvae were transferred onto the leaflets was considered day 0 after infestation (0 DAI). Petri dishes were sealed with Parafilm® M laboratory film (Bemis Company, Inc.) for the first week to prevent the FAW from escaping. For the next three days, Petri dishes with dead FAW larva were replenished with another neonate larva. On the day a Petri dish was restarted with a new FAW larva, that day was considered 0 DAI for that particular plate. The Petri dishes were examined daily to relocate FAW larvae that may have moved off of the leaflets and onto or underneath the filter paper. Daily inspection ensured that FAW larvae would have the opportunity to feed on plant material, and that the filter paper and cotton pad could be moistened if they had become dry. Fresh leaflets were offered every other day for one week, then fresh leaves were offered daily to meet the need of increased larval feeding. The moistened cotton pad and filter paper were changed as the frass from FAW larva accumulated and contaminated the filter paper.

The following parameters were evaluated to study the effect of plant genotype on FAW growth and development: survival, larval weight, larval stage duration, pupation, pupal stage duration,

moth emergence relative to pupation, and moth sex. Low survival, larval weight, pupation, and emergence as well as high larval stage duration and pupal stage duration indicated deceleration of normal FAW growth and development. Sex ratio was an indicator of genotype effect on FAW fitness in which a deviation from a 1:1 (male to female) indicated a genotypic effect of the host plants on FAW sexual dimorphism. Sex ratio was included, since diet and other environmental effects have been found to negatively affect adult insect, including FAW, sex ratios (Bull, 1981; Murúa and Virla, 2004). Biased sex ratio could be utilized as one of the valuable tactics for sustained pest management in multiple cropping systems. FAW survival was documented daily. Larval weight was recorded at 14 DAI. Larval stage duration documented the number of days from first-instar larva stage to pupal formation. Pupation and relative moth emergence described completed pupation and moth emergence relative to the number of pupa formed, respectively. Pupal stage duration denoted the number of days recorded from pupal development to moth emergence. Pupal weight was recorded on the day of complete pupal formation. Moth sex (male or female) was determined after emergence.

Genotyping the diploid species

Cor demonstrated morphological diversity; therefore, we genotyped the diploid species using the Affymetrix Axiom_Arachis2 SNP array (Clevenger *et al*., 2018; Korani *et al*. 2019) consisting of 47,000 features (ThermoFisher Scientific). DNA was extracted by the Qiagen DNeasy Plant mini kit (Qiagen, Germantown, MD). SNP calling was performed with Axiom Analysis Suite (Version 1.2). Genetic markers were grouped into six categories by the software depending on the quality and separation of markers 1) Monomorphic 2) PolyHighResolution 3) NoMinorHom 4) OfftargetVariant 5) CallRateBelowThreshold 6) Other. Only the 5,342 markers in the PolyHighResolution class were used for analysis since the grouping of samples was unambiguous and all the samples passed quality control.

Statistical Analysis

One-way analysis of variance (ANOVA) was performed using JMP software (SAS Institute) to determine the genotype effect on FAW growth and development according to the following parameters: larval weight, larval stage duration, pupal stage duration, and pupal weight. Chi-squared tests were performed with JMP software to determine the genotype effect on the following categorical parameters used to assess FAW development: survival at 8 DAI and 14 DAI (dead or alive), pupation (pupated or did not pupate), relative moth emergence (emerged or did not emerge), and moth sex (male or female). For relative moth emergence, data (emerged or did not emerge) was only included for FAW that pupated. Means of each parameter among the treatments were separated based on the Tukey's Test ($\alpha = 0.05$) results with JMP software. Tukey's Test for binomial data was not supported with JMP software, so RStudio (RStudio, Inc.) was used by running genotype effect on survival at 8 DAI and 14 DAI, pupation and relative moth emergence as a generalized linear model and then Tukey's Test ($\alpha = 0.05$) was performed on the model results. Genotypes with less than four replications for a measurement were excluded from statistical analysis. Therefore, larval stage duration, pupation, pupal weight, pupal stage duration, relative moth emergence, and moth analysis only included eight genotypes for the May 2019 experiment with *Ipa* and *IpaCor*_3 excluded and only four genotypes for the November 2019 experiments with *Cor*, *Dur*, *Ipa*, and *IpaDur*_5 excluded due to being terminated before pupal formation. The data for both the May and November 2019 experiments were still valid, since they were maintained according to experimental design and the controls were continued.

Results

Survival

Significant genotypic effect on FAW survival at 8 DAI and 14 DAI was found for the May 2019 experiment and the November 2019 experiment, but not for the July 2018 experiment (Table 2). However, Tukey-Kramer significant differences between genotypes were not found for neither 8 DAI nor 14 DAI for all three experiments (Table S1). In all three experiments, the cultivated control genotypes had the highest FAW survival (Fig. 1). For the July 2018 experiment (Fig. 1A), the allotetraploid *IpaDur*_2 had the greatest FAW mortality. Only 30% (9 of the 30) of FAW fed exclusively on *IpaDur*_2 survived to the end of the experiment and completed their life cycle. The larval survival curves on *Ipa* and *Dur* were below that of the susceptible cultivated peanut control.

Unlike the other two experiments, the May 2019 experiment (Fig. 1B) had a high mortality of FAW larvae just four days after the onset of the experiment. This is likely due to a trained entomologist performing the July 2018 and November 2019 experiments. However, all allotetraploids had survival curves below the susceptible control. Both accessions of *A. correntina* were

Table 2. ANOVA and chi-squared test output testing the genotype effect on FAW growth and development according to the following parameters: survival at 8 DAI and 14 DAI, larval weight, larval stage duration, pupation, pupal stage duration, pupal weight, relative moth emergence, and moth sex.

Parameter	F or X^2 value	df(n) [a], df(d) [b]	P-value
Survival at 8DAI (July 2018)	8.66	6	0.19
Survival at 8DAI (May 2019)	92.62	9	< 0.0001***[c]
Survival at 8DAI (Nov. 2019)	26.44	7	0.0004***
Survival at 14DAI (July 2018)	11.81	6	0.07
Survival at 14DAI (May 2019)	117.61	9	< 0.0001***
Survival at 14DAI (Nov. 2019)	35.41	7	< 0.0001***
Larval weight (July 2018)	11.14	6, 156	< 0.0001***
Larval weight (May 2019)	23.60	9, 151	< 0.0001***
Larval weight (Nov. 2019)	29.92	7, 205	< 0.0001***
Larval stage duration (July 2018)	12.13	6, 131	< 0.0001***
Larval stage duration (May 2019)	29.60	7, 124	< 0.0001***
Larval stage duration (Nov. 2019)	38.01	3, 92	< 0.0001***
Pupation (July 2018)	20.94	6	0.0019**
Pupation (May 2019)	82.15	7	< 0.0001***
Pupation (Nov. 2019)	38.24	3	< 0.0001***
Pupal Weight (July 2018)	22.68	6, 131	< 0.0001***
Pupal Weight (May 2019)	14.91	7, 124	< 0.0001***
Pupal Weight (Nov. 2019)	84.12	3, 92	< 0.0001***
Pupal stage duration (July 2018)	2.66	6, 117	.019*
Pupal stage duration (May 2019)	8.92	7, 113	< 0.0001***
Pupal stage duration (November 2019)	1.32	3, 79	0.27
Relative moth emergence (July 2018)	11.45	6	0.077
Relative moth emergence (May 2019)	6.14	7	0.052
Relative moth emergence (Nov. 2019)	10.66	3	< 0.014*
Moth sex (July 2018)	7.32	6	0.29
Moth sex (May 2019)	11.92	7	0.10
Moth sex (Nov. 2019)	4.29	3	0.23

[a]The df(n), degrees of freedom of the numerator, is based on the number of plant genotypes tested

[b]The df(d), degrees of freedom of the denominator, is based on the total number of replicates, in which a replicate is one plate with one FAW, for all plant genotypes tested

[c]*P < .05. **P < .01. ***P < .001

similar to the susceptible control, although *Cor*9530 lost five more FAW larvae (17% more) than *Cor*9548.

In the November 2019 experiment (Fig. 1C), *Ipa*, *Dur*, *Cor*, and *IpaDur*_5 had to be terminated early due to insufficient plant material to sustain the FAW feeding. However, FAW larvae fed on susceptible controls showed almost identical survival curves as the previous two experiments. The FAW larva survival curve on *Cor*9530 closely followed survival curves of the susceptible controls. *IpaCor*_4, a progeny line from *IpaCor*_1 that showed high FAW mortality in the May 2019 experiment (Fig. 1B), also had a survival curve similar to the controls. *Ipa* and *Dur* had similar survival curves to the July 2018 experiment (Fig. 1C) until the treatments were terminated. *IpaCor*_5 (Fig. 1C), a progeny line from *IpaCor*_2 (Fig. 1B) that had high FAW mortality in the May 2019 experiment, had the greatest FAW mortality in the November 2019 experiment.

Larval Weight

Genotype had a significant effect on larval weight in all of the experiments (Table 2). All five *IpaDur*^{4x} allotetraploid plants significantly reduced FAW larval weight compared to the cultivated peanut genotypes included as experimental controls (Fig. 2). For the July 2018 experiment, the allotetraploid *IpaDur*_2 that had the greatest FAW mortality also had the numerically lowest larval weight (Figs. 1A, 2A). As for *IpaCor9530*^{4x}, three out of five tested allotetraploid plants (*IpaCor*_1, *IpaCor*_4, and *IpaCor*_5) significantly suppressed larval weight (Figs. 2B, C). The other two *IpaCor9530*^{4x} plants (*IpaCor*_2 and *IpaCor*_3) also reduced larval weight, yet the level of reduction did not show statistical significance when compared to the cultivated peanut controls (Fig. 2B). This is partly due to high mortality of FAW larvae in the May 2019 experiment (Fig. 1B), which led to small sample size, and affected statistical power for larval weight data (Fig. 2B). For example, while the larval

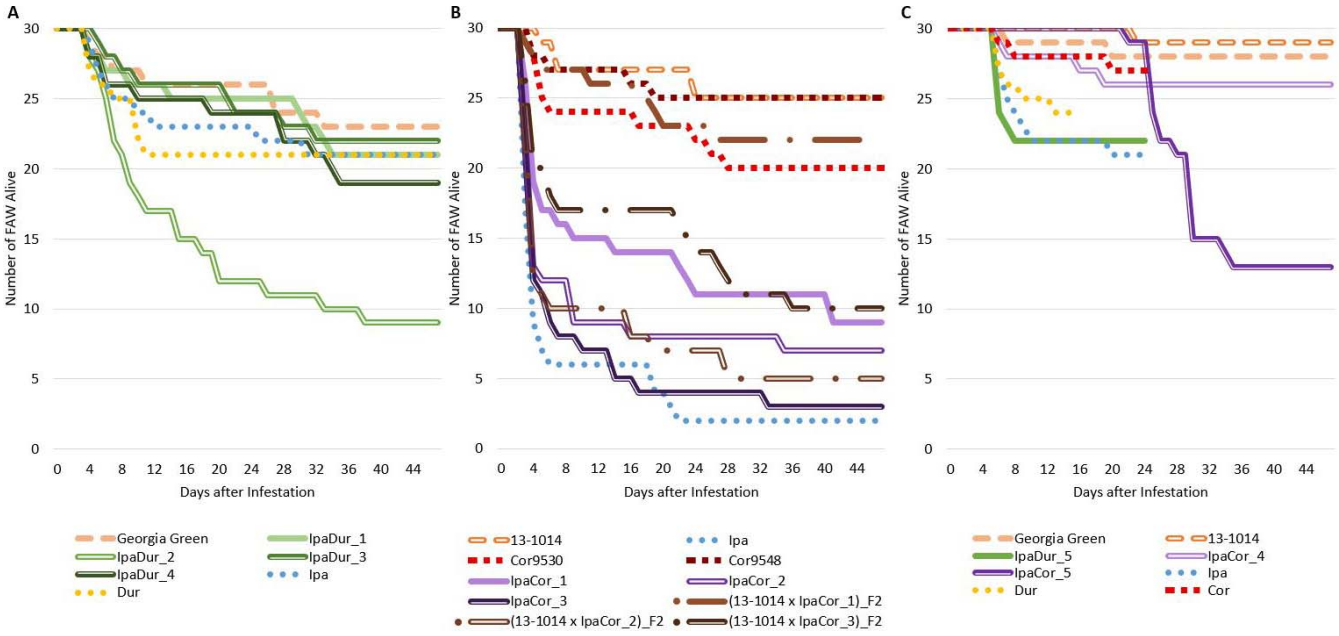

Fig. 1. Survival of FAW throughout the life cycle during the experimental period. (A) July 2018 Experiment. (B) May 2019 Experiment. (C) November 2019 Experiment.

weight on *IpaCor*_1 was 35% of the control and found to be significantly different, the *Ipa* larval weight was only 13.5% of the control but not significantly different due to the small sample size caused by mortality of FAW larvae on *Ipa* leaves. When the two allotetraploid lines were tested side-by-side in the November 2019 experiment (Fig. 2C), *IpaDur*_5 demonstrated a stronger level of FAW resistance by reducing FAW larval weight to a greater extent than *IpaCor9530*_4 and *IpaCor*9530_5.

Diploid parents of both allotetraploid lines were included in this study to determine the source of FAW resistance. Compared to the cultivated peanut control, *Ipa* significantly suppressed FAW weight gain in the July 2018 and November 2019

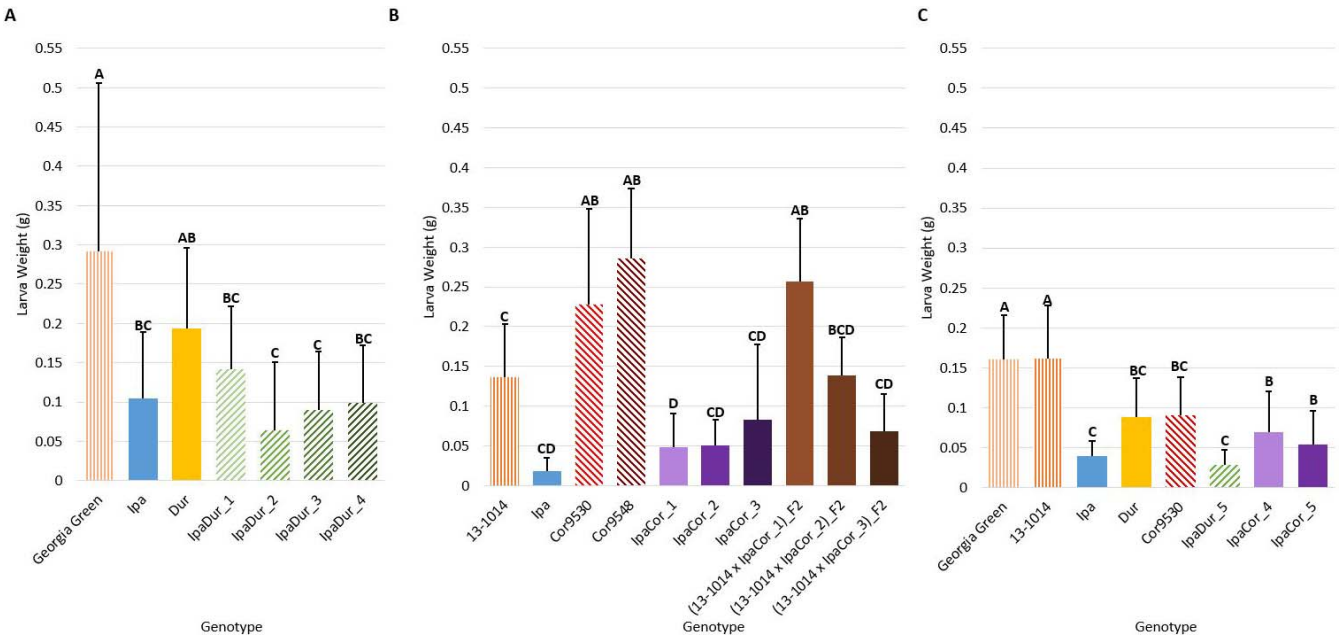

Fig. 2. Larva weight on day 14 after feeding on different peanut leaflets. (A) July 2018 Experiment. (B) May 2019 Experiment. (C) November 2019 Experiment. Error bars represent standard error. Tukey's HSD significance levels were calculated within each experiment, so these significance groupings cannot be compared between the three experiments. Genotypes within an experiment with the same Tukey's HSD letter are not significantly different ($\alpha = 0.05$).

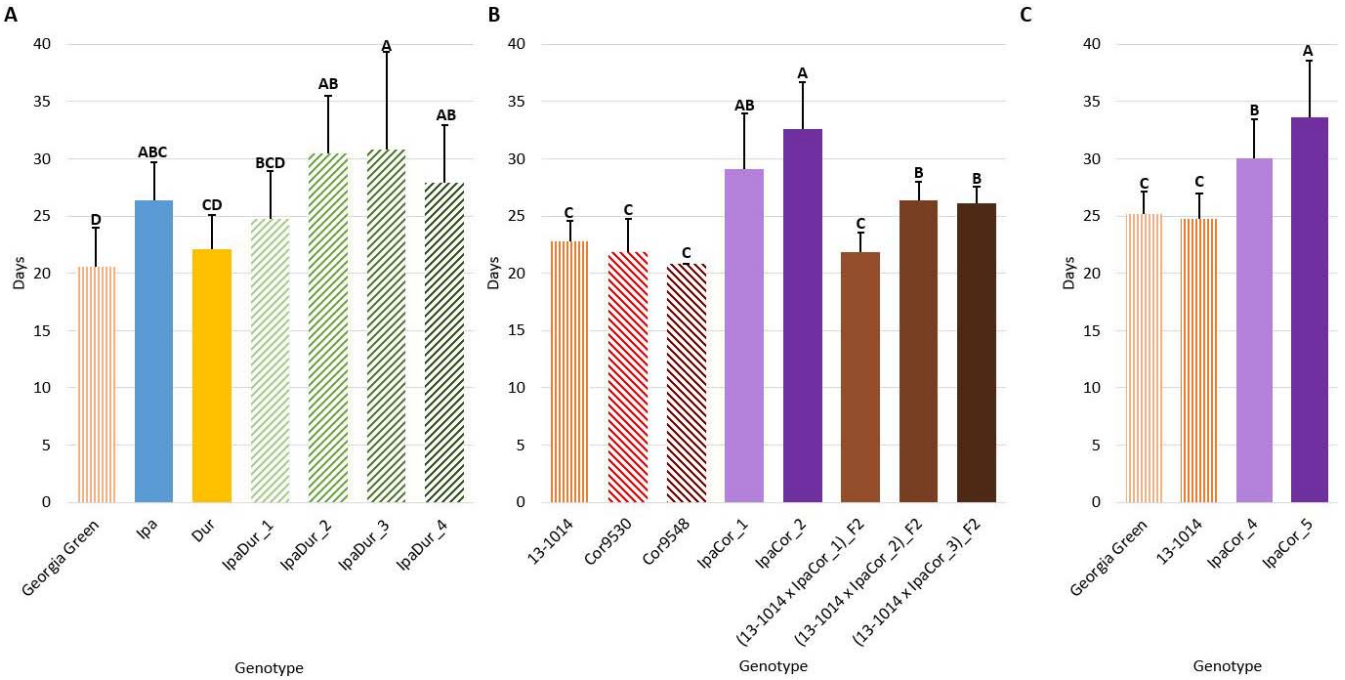

Fig. 3. Larval stage duration. (A) July 2018 Experiment. (B) May 2019 Experiment. (C) November 2019 Experiment. Error bars represent standard error. Tukey's HSD significance levels were calculated within each experiment, so these significance groupings cannot be compared between the three experiments. Genotypes within an experiment with the same Tukey's HSD letter are not significantly different ($\alpha = 0.05$).

experiments (Figs. 2A, C). *Dur* also significantly reduced the weight of FAW larvae but to a numerically lesser extent than that of *Ipa* in the November 2019 experiment. Conflicting results were found between the May 2019 (Fig. 2B) and November 2019 (Fig. 2C) experiments for *Cor*9530. A significant, positive effect on larval weight was observed in the May 2019 experiment (Fig. 2B) and a significant suppressive effect was found in the November 2019 experiment (Fig. 2C). *Cor*9548 was tested only in one experiment (Fig. 2B) and it was found to increase larval weight gain. A high level of genetic heterogeneity was found in *Cor*9530 (Table S2). Among the three genotyped *Cor*9530 plants, 1,259 out of 5,342 total markers (23.5%) were found to be polymorphic. On the contrary, only 23 (0.4%) and 27 (0.5%) polymorphic markers were found with *Ipa* and *Dur*, respectively. Therefore, the conflicting feeding response on *Cor*9530 could be due to the genetic diversity within this accession. In the May 2019 experiment (Fig. 2B), three 13-1014 x *IpaCor* F_2 plants were found to be segregating for larval weight. The (13-1014 x *IpaCor*_1)_F_2 plant demonstrated a significantly greater increase in larval weight compared to the cultivated control. The (13-1014 x *IpaCor*_2)_F_2 plant also showed a similar effect on larval weight compared to the cultivated control. The (13-1014 x *IpaCor*_3)_F_2 plant demonstrated high suppression of FAW growth similar to the *IpaCor9530*^{4x}.

Larval Stage Duration

Genotype had a significant effect on larval stage duration in all of the experiments (Table 2). In all three experiments, the cultivated controls had the shortest larval stage duration (Fig. 3). Compared to the cultivated control, *Ipa* significantly reduced larval growth and development by extending larval stage duration in the July 2018 experiment (Fig. 3A). Likewise, all allotetraploids significantly extended larval stage duration, except for *IpaDur*_1 in the July 2018 experiment (Fig. 3A). *IpaCor*_5, the allotetraploid with the greatest FAW mortality in the November 2019 experiment (Fig. 1B), significantly outperformed *IpaCor*_4 by greatly extending larval stage duration (Fig. 4B). While *Ipa* significantly extended larval stage duration, *Dur, Cor*9530, and *Cor*9548 did not differ significantly from the cultivated controls.

Pupation

Genotype had a significant effect on pupation in all of the experiments (Table 2). For the July 2018 and May 2019 experiments, Tukey-Kramer significant differences between genotypes were not found; however, 13-1014 had significantly more pupated FAW than *IpaCor*_5 (Table S1). Across all the experiments, the cultivated controls had the highest number of FAW to pupate (Fig. 4). For the July 2018 experiment, *IpaDur*_2, which had the highest FAW mortality (Fig. 1A), also had the fewest FAW to pupate (Fig. 4A). For the May 2019 experiment (Fig. 4B), pupation was heavily

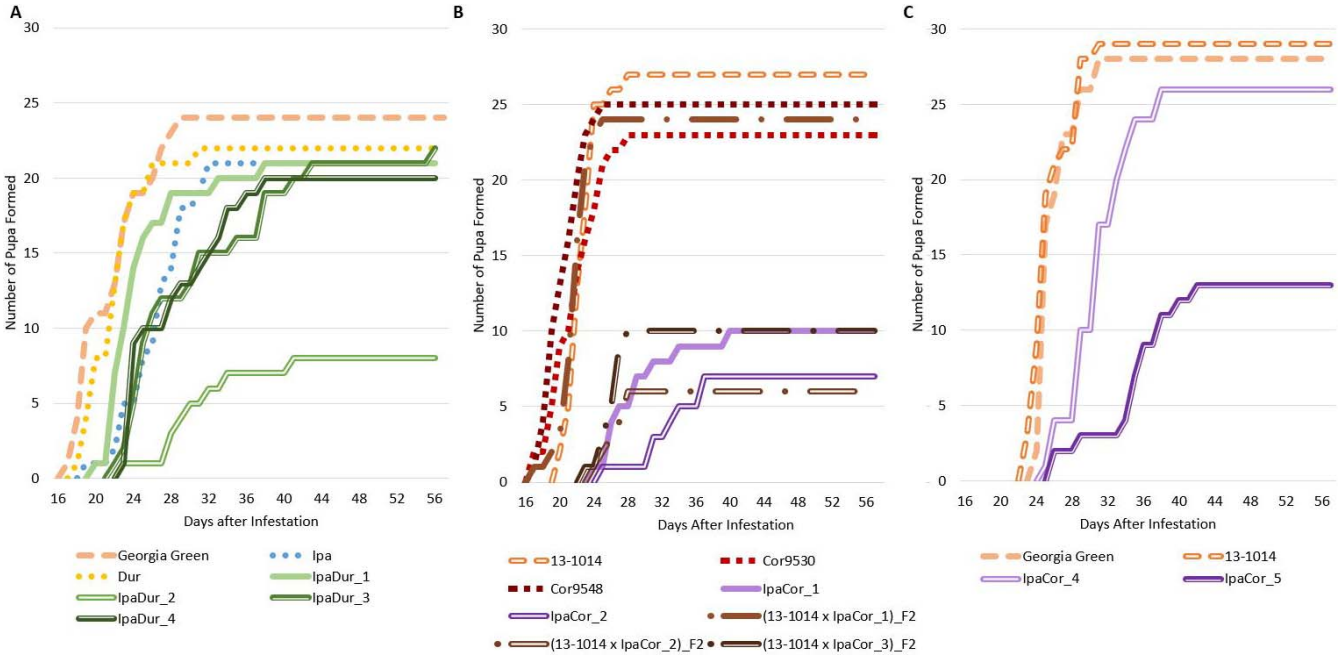

Fig. 4. Pupation. (A) July 2018 Experiment. (B) May 2019 Experiment. (C) November 2019 Experiment.

influenced by FAW mortality early in the experiment. Despite this artifact, all allotetraploids had a lower number of FAW to pupate than the cultivated control. Both *Cor*9530 and *Cor*9548 had a similar number of FAW to pupate as compared to the cultivated control. Line (13-1014 x *IpaCor*_1)_F_2 also had a similar number of FAW to pupate as compared to the cultivated controls, while (13-1014 x *IpaCor*_2)_F_2 and (13-1014 x *IpaCor*_3)_F_2 had much lower numbers of FAW to pupate as compared to the cultivated controls. For the November 2019 experiment, *IpaCor*_4 had a similar number of FAW to pupate as the cultivated controls (Fig. 4C), while *IpaCor*_5 had significantly less pupa form as compared to 13-1014 (Table S1).

Pupal Weight

Genotype had a significant effect on average pupal weight across all experiments (Table 2). All allotetraploids, except *IpaCor*_1 in the May 2019 experiment, had significantly lower pupal weight than the cultivated controls (Fig. 5). *IpaDur*_3 and *IpaDur*_4 had significantly lower pupal weight than *IpaDur*_1 (Figs. 5A, C). *IpaCor*_2 had the lowest pupal weight of all the allotetraploids in the May 2019 experiment (Fig. 5B). *IpaCor*_5, the progeny line of *IpaCor*_2, had the lowest pupal weight in the November 2019 experiment (Fig. 5C). *Ipa*, *Dur*, and *Cor*9548 all had significantly lower pupal weight than the respective cultivated control. However, *Cor*9530 was not statistically different from the cultivated control in the May 2019 experiment (Fig. 5B).

Pupal Stage Duration

Genotype had a significant effect on pupal stage duration in the July 2018 experiment and the May 2019 experiment, but not in the November 2019 experiment (Table 2). Across all three experiments, *Ipa*, *Dur*, *Cor*9530, and *Cor*9548 were not significantly different from the cultivated controls (Fig. 6). However, *IpaCor*_1 and (13-1014 x *IpaCor*_2)_F_2 had significantly longer pupal stage duration than the cultivated control, indicating these materials impeded pupal stage development (Fig. 6B).

Relative Moth Emergence

Genotype had a significant effect on relative moth emergence in the November 2019 experiment but not the July 2018 or May 2019 experiments (Table 2), and Tukey-Kramer significant differences between genotypes for relative moth emergence were not found for any of the three experiments (Table S1). All of the allotetraploids, except for *IpaCor*_2 and *IpaCor*_4 (Fig. 7B, C), supported less relative moth emergence than the cultivated controls (Fig. 7). Allotetraploids had a high number of aborted pupae with the highest being 38.5% (5 out of 13 pupa) for *IpaCor*_5, then 25% (5 out of 20 pupa) for *IpaDur*_4, and 18.9% (4 out of 22) for *IpaDur*_3 (Fig. 7A, C). In comparison, Georgia Green had 4.2% (1 out of 24) and 7.1% (2 out of 28) aborted pupa in the July 2018 and November 2019 experiments, respectively (Fig. 7A, C). *Arachis hypogea* 13-1014 had 7.1% (2 out of 28) and 6.9% (2 out of 29) aborted pupa in the July 2018 and November 2019 experiments, respectively

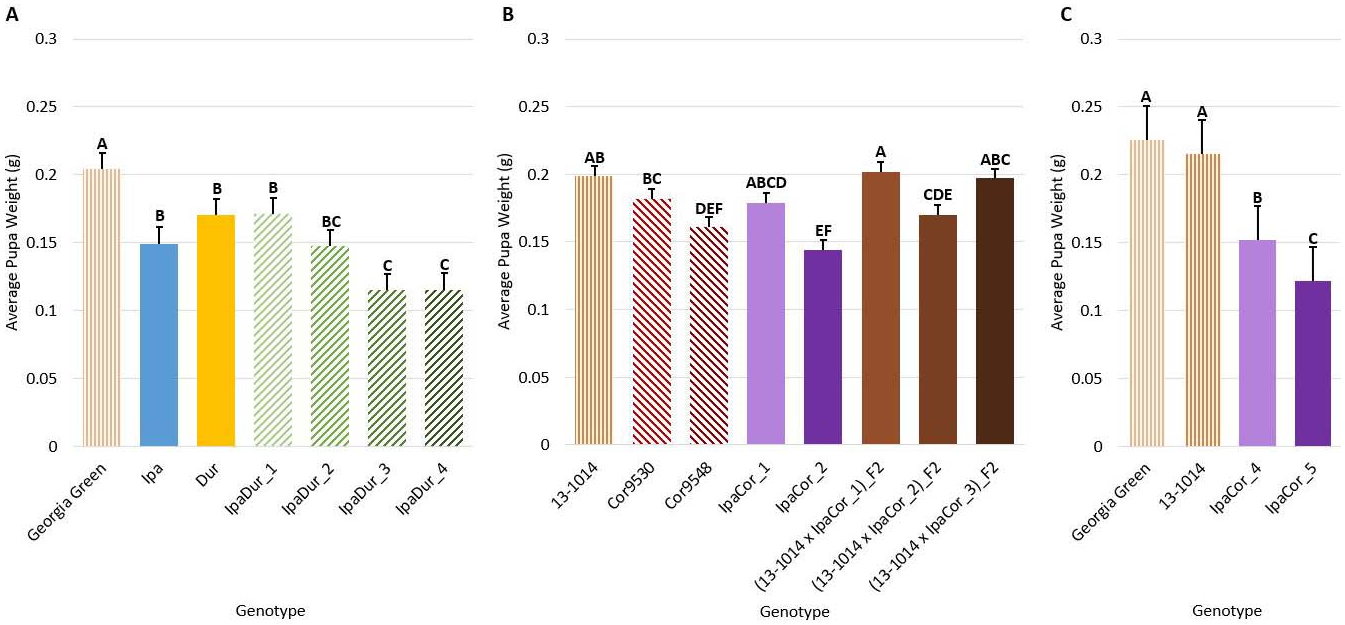

Fig. 5. Pupal weight. (A) July 2018 Experiment. (B) May 2019 Experiment. (C) November 2019 Experiment. Error bars represent standard error. Tukey's HSD significance levels were calculated within each experiment, so these significance groupings cannot be compared between the three experiments. Genotypes within an experiment with the same Tukey's HSD letter are not significantly different ($\alpha = 0.05$).

(Fig. 7A, C). The overall low number of 8 moths formed on *IpaCor*_5, a progeny line of *IpaCor*_2, was due to early FAW mortality and high pupa abortion (Figs. 1B, 7B). All larvae fed on *Ipa*, *Dur*, *Cor*9530, and *Cor*9548 showed similar relative moth emergence as compared to the cultivated controls. Also, larvae fed on *Cor*9530 showed similar relative moth emergence as compared to the larvae fed on *Cor*9548 (Fig. 7B). Lastly, the (13-1014 x *IpaCor*)_F_2 plants all had similar relative moth emergence when compared to the control (Fig. 7B).

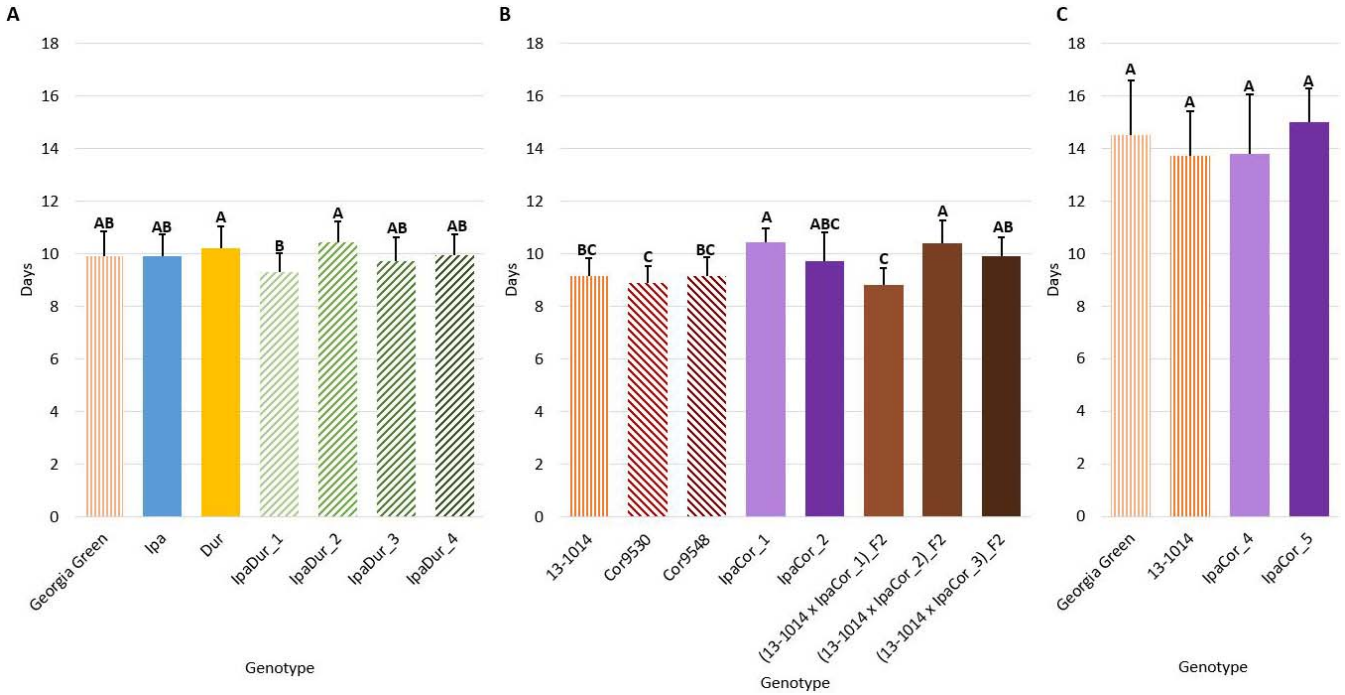

Fig. 6. Pupal stage duration. (A) July 2018 Experiment. (B) May 2019 Experiment. (C) November 2019 Experiment. Error bars represent standard error. Tukey's HSD significance levels were calculated within each experiment, so these significance groupings cannot be compared between the three experiments. Genotypes within an experiment with the same Tukey's HSD letter are not significantly different ($\alpha = 0.05$).

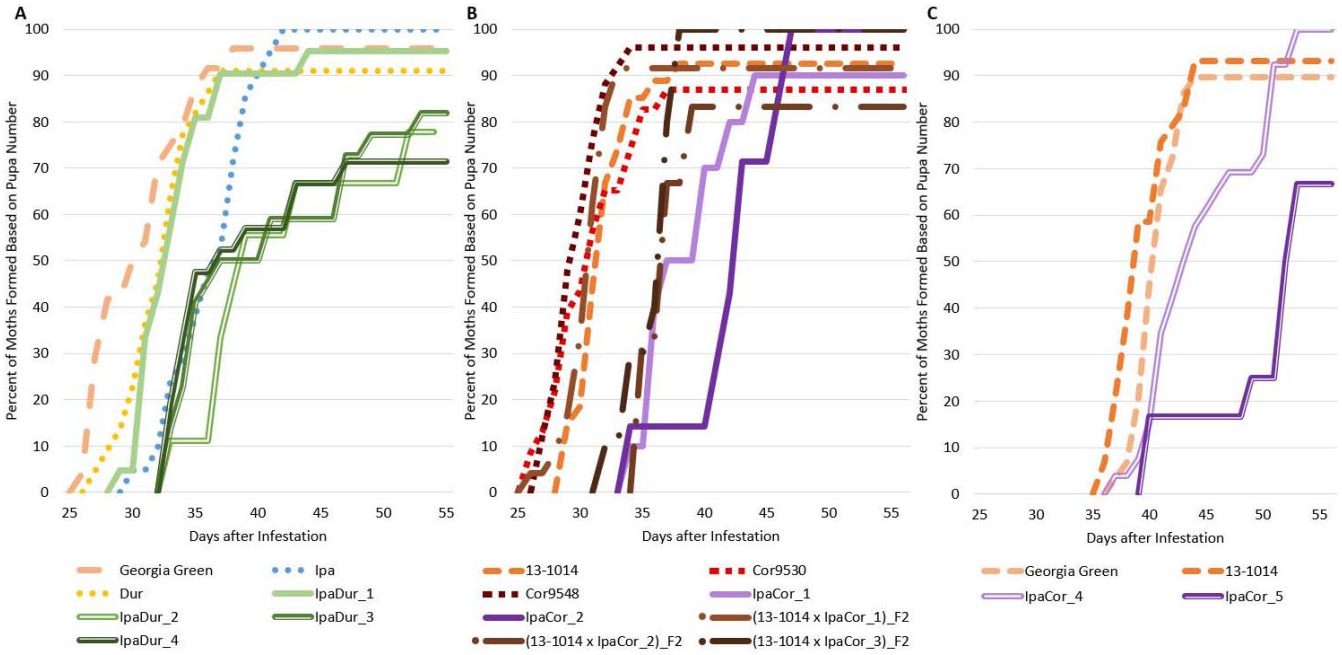

Fig. 7. Percent of moths formed based on pupa number. (A) July 2018 Experiment. (B) May 2019 Experiment. (C) November 2019 Experiment.

Moth Sex

Genotype did not have a significant effect on moth sex in the July 2018, May 2019, nor November 2019 experiment (Table 2).

Discussion

The wild peanut-derived allotetraploid genotypes showed promise as sources for FAW resistance for peanut breeding programs, since all *IpaDur*^{4x} and *IpaCor*9530^{4x} allotetraploids significantly reduced larval weight (except *IpaCor*_2 and *IpaCor*_3), increased pupal stage duration (except *IpaDur*_1), and decreased pupal weight (except *IpaCor*_1) as compared to the controls. All allotetraploid genotypes showed lower survival and pupation than the cultivated genotypes evaluated as well as lower relative moth emergence (except *IpaCor*_2 and *IpaCor*_4). The most promising allotetraploid line, *IpaDur*_2, had the lowest larval weight and the greatest FAW mortality in the July 2018 experiment. This was further supported by *IpaDur*_5, which significantly reduced larval and pupal weight in the November 2019 experiment. *IpaCor*_2 and its progeny *IpaCor*_5 were shown as a promising line as they both suppressed FAW growth in the May and November 2019 experiments. Since segregation for FAW resistance in the allotetraploids was found, further selections for FAW resistance in individual lines will be necessary for effective utilization of these materials in a breeding program.

Primarily *Ipa*, but also *Dur*, were shown to be donor sources for FAW resistance, by reducing larval and pupal weight, survival and pupation. The conclusion that *Ipa* is valuable as a source for FAW resistance confirms a previous report (Yang *et al*., 1993). However, a 79% mortality rate at 8 DAI was reported for *A. ipaensis* in a similar bioassay with five wild *Arachis* species and *A. hypogaea* (Yang *et al*., 1993). The bioassay did differ in the use of terminal buds from field-grown plants rather than expanded leaves from greenhouse-grown plants. Two of our three experiments showed generally higher survival for *Ipa* with 8 DAI mortality rates of 16.7%, 80%, and 20% for the July 2018, May 2019, and November 2019 experiments, respectively. The high mortality of FAW fed on *Ipa* leaves in the May 2019 experiment may be an artifact of this experiment having overall higher FAW mortality than the other two experiments (Fig. 1), influenced by a trained entomologist performing the July 2018 and November 2019 experiments. Overall, the mean *Ipa* mortality rate was 38.9% across the three experiments. However, the low larval weight of *Ipa* supports the previous conclusions (Yang *et al*., 1993). Another FAW bioassay study (Lynch *et al*., 1981) tested 14 wild *Arachis* species, not including *Ipa* and *Dur*, and recommended *Cor* (PI 261870) as a source for FAW resistance. *Cor* (PI 261870) impeded FAW survival and development and also showed antixenosis in a free-choice preference test. We tested *Cor*9530 and *Cor*9548 instead of *Cor* (PI 261870),

because *Cor* (PI 261870) seeds were not available. Unlike the previously reported results, conflicting results were found regarding the FAW resistance in *Cor*9530 plants tested in these experiments. Genotyping detected a high level of SNP variation within *Cor9530,* suggesting this diploid species accession is highly heterogeneous which may explain the contradictory bioassay results of FAW resistance within this species. In addition, *IpaCor*_1, *IpaCor*_2, and *IpaCor*_3 were derived from crosses between *Ipa* and different *Cor9530* individuals, so genetic difference between these lines can be ascribed to parental heterogeneity or genetic segregation in the allotetraploids.

This study builds upon previous reports by focusing on allotetraploids that are cross compatible to *A. hypogaea* and are therefore valuable to breeding programs, instead of focusing solely on diploid, wild *Arachis* species. In the May 2019 experiment, varied levels of FAW resistance were detected among the three tested F_2 lines from 13-1014 x *IpaCor9530*^{4x} cross. The (13-1014 x *IpaCor*_1)_F_2 plant had an even greater larval weight than *A. hypogaea* 13-1014, while the (13-1014 x *IpaCor*_3)_F_2 plant was similar to the allotetraploids, suggesting the FAW resistance from *IpaCor9530*^{4x} segregated in the F_2 populations. An expanded study of a population derived from the tested (13-1014 x *IpaCor*)_F_2 materials is needed to determine the inheritance pattern and conduct genetic mapping of FAW resistance QTL.

The major limitation of this study is that all three experiments were confined to *in vitro* bioassays using excised peanut leaflets. Due to the required labor for these bioassays that included daily examination of each plate for 44 days per experiment, only a limited number of genotypes could be tested. A progression to this study would be to confirm these results in a field trial and then to map FAW resistance QTL in a mapping population. However, it is difficult to maintain a peanut field with FAW infestation in the southeastern U.S. because of the abundance of natural insect enemies in the peanut fields throughout the growing season and the sporadic nature of FAW as a pest on peanut plants. However, a field study could be performed in collaboration with partners in Africa, where FAW is a newly invasive pest and FAW pressures are high.

Conclusions

This study built upon previous reports by testing FAW resistance in the unique allotetraploids instead of just wild, diploid *Arachis* species alone. The allotetraploids examined in this study showed strong FAW resistance, making them more useful than peanut cultivars previously found to have only moderate levels of FAW resistance. Furthermore, these allotetraploids are cross-compatible with peanut cultivars, making this resistance accessible for peanut breeders. These FAW resistant allotetraploids will be shared with breeding programs in the United States and Africa so that FAW resistance can be introgressed into elite peanut cultivars to effectively reduce the impact of the FAW (as an invasive pest) on peanut production in countries where growers have limited access to pesticides. At the same time, FAW resistant peanut cultivars can protect yields in the United States, increase yields in regions with limited use of pesticides, decrease reliance on pesticides, and promote organic peanut production and sustainable agriculture in general.

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