INFLUENCE OF NITROGEN LEVELS ON COTTON PLANT/INSECT INTERACTIONS IN A CONSERVATION TILLAGE SYSTEM

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REFERENCE: J. E. Hook (ed.) *Proceedings of the 22nd Annual Southern Conservation Tillage Conference for Sustainable Agriculture.* Tifton, GA. 6-8 July 1999. Georgia Agriculture Experiment Station Special Publication 95. Athens, GA.

Abstract. Plants have indirect defenses against herbivores through the attraction of the third trophic level to damaged plants that have been induced to produce and emit volatile chemical signals. These defenses can increase plant fitness, but recent studies indicate that nitrogen levels can effect a plant's ability to produce them. This study tests the effects of various nitrogen levels in a cotton field conservation tilled with plants previously damaged by *Spodoptera exigua* on the abundance of insect species, fruit production and damage, and total plant yield. Nitrogen was applied at 0, 30, 60 & 120 lb/acre in a conservation-tilled cotton field planted with a winter cover crop of Crimson clover, with 10 plants per plot damaged by *S. exigua* larvae. Whole plants were sampled twice during the season. There was a general pattern of increasing numbers of *Helicoverpa zea* and *Heliothis virescens* eggs and larvae, and lacewing eggs, larvae and pupae with increasing nitrogen, and previous plant damage had an effect on the number of eggs, larvae and pupae only at high nitrogen levels. Total fruit production and damage was highest in the plots with the highest nitrogen, but fruit production and damage was not influenced by previous plant damage by *S. exigua*. Yields across all nitrogen levels were not significantly different. The oviposition preference of insects on previously damaged plants at high nitrogen levels, may indicate that plant signals have been altered by nitrogen rates in such a manner that the pest perceives a weakened plant and the predator perceives higher numbers of prey.

INTRODUCTION

A total system approach to pest management requires that we consider crop plants as active components of multi-trophic interactions. Plants can have both intrinsic (direct) defenses, as well as extrinsic (indirect) defenses against herbivores and pathogens and these defenses can be affected by plant nutrition and other environmental factors (Bernays & Chapman 1994). Examples of intrinsic defenses are production of toxins or digestibility reducers, or through physical defense by trichomes or toughness, or by a combination of the two, as with glandular trichomes or resins. Extrinsic defenses are when a plant benefits from the natural or applied enemies of herbivores (Price 1986). Extrinsic defenses may be brought about by the attraction of the third trophic level to damaged plants that have been induced to produce and emit volatile chemical signals (Agrawal 1998; Alborn et al. 1997; Cortesero et al. 1997; Paré & Tumlinson 1997a 1997b; Röse et al. 1998; Tumlinson et al. 1992; Turlings et al. 1990, 1991). In the only field test of induced resistance to herbivores and plant fitness, Agrawal (1998) found that previous damage by herbivores decreased subsequent herbivory and enhanced the seed mass of radishes. The previous study did not examine plant nutrition effects on herbivory and plant fitness, and recent studies indicate that these effects can have a large effect on a plant's ability to produce direct and indirect defenses against herbivory (Cortesero et al. unpublished data). In their study, Cotersero et al., found that high nitrogen levels decreased the release of induced volatiles of damaged cotton plants and the subsequent attraction to these plants by *Microplitis croceipes* (Cresson) a parasitoid of major cotton pests, *Helicoverpa zea* (Boddie) and *Heliothis virescens* (Boddie). In addition, cotton plants maintained their ability to produce antifeedants under all nitrogen levels tested, bu the high nitrogen plants received significantly higher leaf area damage than nitrogen applied at lower levels. Thus, awareness of plant effects on multi-trophic systems is essential in integrating plant breeding and biological control using natural enemies.

Our objective is to extend the study of Agrawal (1998) to a cotton system and to include plant nutrition with previous plant damage to test the their effects on plant fitness and the presence of plant-feeding insects and natural enemy species. Specifically, we will test the effects of various nitrogen levels in a cotton field conservation tilled with plants previously damaged and not previously damaged by *Spodoptera exigua* (Hübner) on the abundance of pests and predators, fruit production and damage, and total plant yield. A more focused study involving fitness effects of species showing strong response to these treatments will be the subject of subsequent field studies.

MATERIALS AND METHODS

A field located in the coastal plain region of southern Georgia, was planted in cotton, *Gossypium hirsutum* L. var. Deltapine acala 90 and sampled from July through September 1998. The field was conservation-tilled with a winter cover crop of Crimson clover (*Trifolium incarnatum* L. 'Dixie'). The field was previously treated with herbicides, Butoxone 175 and Gramoxone and fertilized with NPK 10-10-10 3x at 300 lb/acrecre. Cotton was planted on May 29 1998, no-till into Crimson clover with seed spacing at 3 seeds every 9.5 in.

Experimental Design

A set of experiments were conducted to test the effect of various nitrogen levels and damage of plants by the herbivore, *Spodptera exigua* Hübner on fruit production, damage of fruits and the presence of pest and natural enemy species. Nitrogen was applied twice at 0, 30, 60 & 120 lb/acre with ammonium nitrate (34-0-0). The nitrogen treatment was replicated 4 times resulting in 16 plots each 36 ft. long x 36 ft. wide. Sampled plants were separated from bordering plots and the edge of the field by 3 rows of cotton. Within each plot, 10 randomly chosen plants were designated for *S. exigua* damage and another 10 plants were not damaged. A damaged plant was obtained by placing 3-4 late 3rd and early 4th instar larvae of *S. exigua* , reared on artificial diet based on pinto beans as described by King & Leppla (1984) on 2 primary leaves from the middle of the plant and allowing feeding for 3 d. Larvae were held on the leaf and protected from predators by enclosing the leaf with a cotton bag (7 in. x 7 in.) with the opening gently secured to the petiole with a pipe cleaner. A total of 640 damaged and undamaged plants across 4 nitrogen levels and 4 replicates were sampled by 4 and 2 people on the first sampling and second date, respectively. Whole plant sampling for fruit production and plant damage occurred on July 22nd, 4 d prior to plant damage, and again on August 18th, 23 d after the larvae were placed on the plants. Whole plant sampling of insects occurred from July 31st to August 3rd, 5 days after the larvae were placed on the plant and again from August 20th to August 28th. Harvest occurred on November 6, 1998 and cotton yields were determined.

Sampling

Total fruit production and fruit damage was determined by counting the number of squares and bolls produced and those that were damaged. Percent plant damage was determined by taking the ratio of the total number of damaged squares and bolls to the total number of squares and bolls produced per plant. The total number of pests and beneficial insect species present was determined by counting the number of *Helicoverpa zea*, *Heliothis virecsens*, *Spodoptera exigua* and larvae, aphids, lacewing (*Chysoperla* & *Chrysopa* spp.) eggs, larvae and pupae, fire ants (*Solenopsis* spp.), coccinellid spp. adults, larvae, pupae and eggs, spiders and adult parasitoid sp. on each plant. Sampling for syrphid fly (*Syrphus* sp.), big-eyed bugs (*Geocoris* sp.), damsel bugs (*Nabis* spp.), assassin bugs (*Sinea diadema* and *Zelus* spp.), minute pirate bug (*Orius* spp), thrips (*Scolothrips sexmaculatus*) and stinkbug (*Podisus maculiventris*) predators and pests were also carried out but they were either absent or their numbers were so low that we do not report their presence.

Total nitrogen content of petioles and blades of cotton plants within each nitrogen treatment was determined by sampling plants 3 x during the study. Sampling occurred on July 22nd, August 21st and September 10th. Within each plot, 2 primary leaves and the petiole were removed from the middle of a randomly chosen cotton plant. A total of 16 samples on each date were obtained for mineral and nutrient analyses. A soil sample from each plot was obtained on August 7th for determination of total soil nitrates.

Statistical Analysis

The design was a randomized complete block with date classified as a super block. The effects of date, replication, nitrogen level, and their interaction on the percent nitrogen of petiole and blade samples after arcsine square root transformation were tested with GLM (SAS, SAS Institute, 1985). The effects of date, replication, nitrogen level, and their interaction on the total number of squares and bolls produced, the number of damaged squares, the number of damaged bolls, plant height and the percent damage of squares and bolls after arcsine square root transformation were tested with GLM (SAS, SAS Institute, 1985). Replication was nested within date and type III sums of squares were used for the error. The effects of replication, nitrogen level, plant damage and the interaction between nitrogen and plant damage on the total number of *H. zea*, *H. virescens* and *S. exigua* eggs (not hatched) and larvae, lacewing eggs (not hatched), larvae and pupae, aphids, fire ants, spiders and adult parasitoids were tested with GLM (SAS, SAS Institute, 1985). The number of aphids, and *H. zea* and *H. virescens* eggs were log-transformed to stabilize the variance. The effects of nitrogen on plant yield were tested with GLM (SAS, SAS Institute, 1985).

RESULTS

Blade, Petiole and Soil Nitrogen

The amount of nitrogen applied and the date of sampling significantly influenced the mean percent of leaf nitrogen (DF = 6, MS = 0.001, F = 2.73, $P < 0.040$). Significantly higher nitrogen was found in blades on the first sampling date (Fig. 1A). On the first and last sampling dates, the nitrogen level of the blades did not differ among nitrogen plots (Fig 1A). However, on the second sampling date, significantly higher leaf nitrogen was found in the plots with no nitrogen than those in the plots where 120 lb/acre nitrogen had been applied leading to the significant date by nitrogen interaction (Fig. 1A). On the second sampling date, leaf nitrogen was significantly lower in the highest nitrogen plots than leaf nitrogen from all other plots and for all sampling dates (Fig. 1A).

The mean percent of petiole nitrogen was significantly influenced by the date of sampling (DF = 2, MS = 0.352, F = 460.92, P < 0.001). Significantly higher petiole nitrogen was found on the second sampling date (Fig 1B).

Soil nitrates were significantly influenced by nitrogen treatment (DF = 3, MS = 142.67, F = 4.94, P < 0.028). Significantly higher soil nitrate levels were found in plots with 60 and 120 lb/acre than those from plots with 0 and 30 lb/acre nitrogen applied (Fig 1C).

The nitrogen level of plots and date significantly influenced plant height (Table 1). Plants in the plots with the highest nitrogen applied were significantly taller than plants in all other plots on both sampling dates (Fig. 1D).

Plant Damage and Yield

The total amount of fruit (squares and bolls) on the cotton plants was influenced by the amount of nitrogen applied (Table 1). Significantly more fruit was found on plants in plots with 120 lb/acre than 30-lb/acre nitrogen applied (Fig. 2A). Previous plant damage had no effect on the total amount of fruit on plants (Table 2).

The nitrogen applied to the plots significantly influenced the total number of damaged squares (Table 1, Fig. 2B). Significantly higher numbers of squares were damaged on plants in the plots with 120 lb/acre nitrogen applied than plants within plots from the same date with zero nitrogen applied, and the plants within plots from the first sampling period with 30 lb/acre nitrogen (Fig. 2B). Previous plant damage had no effect on the number of damaged squares within the second sampling date (Table 2).

The total number of damaged bolls on plants was significantly influenced by the sampling date and the nitrogen applied to the plots (Table 1). Very few bolls were damaged in the first sampling period because few bolls were present (Fig.2C). However, significantly higher numbers of bolls were damaged by the second period on the plants with 120 lb/acre nitrogen applied (Fig. 2C). Previous plant damage had no effect on the number of damaged bolls on the plants (Table 2).

The date of sampling and the nitrogen applied to plots significantly influenced the total percent of fruit damaged (Table 1). The proportion of damaged fruit was significantly higher on the first date in plots with no nitrogen applied than those plants of the same date with 30 lb/acre and all plots of the second date (Fig. 2D). On the second sampling date, a significantly higher proportion of the fruit was damaged on plants in plots with 120 lb/acre nitrogen applied (Fig. 2D). Previous plant damage had no effect on the percent of the fruit damaged (Table 2).

Plant yield was not significantly influenced by the nitrogen applied to the plots (DF = 3, MS = 13868.51, F = 0.44, P > 0.726).

Insects

The total number of *H. zea* and *H. virescens* eggs and larvae that were found on cotton plants was significantly influenced by the date of sampling and previous plant damage (Table 3). More eggs and larvae were found on the second sampling date on previously damaged plants but previous plant damage had no effect on the first sampling date (Fig. 3A & 3B). There was also a significant effect of date and nitrogen applied on the number of eggs found on plants (Table 3). Significantly more eggs were found on plants in plots with 120 lb/acre than 30 lb/acre nitrogen applied on the second sampling date and than all plots on the first sampling date (Fig. 3C). Pooling the 0 & 30 lb/acre and 60 & 120 lb/acre nitrogen treatments on the second sampling date indicate that significantly more *H. zea* and *H. virescens* eggs were oviposited on the high nitrogen plants that had been previously damaged (Fig. 3D, MS = 0.54, DF = 1, F = 4.16, P < 0.043 for the interaction between nitrogen and damage treatments). There was no significant effect of nitrogen and previous plant damage on the number of *S. exigua* egg masses or larvae (For egg masses: MS = 0.08, DF = 3, F = 2.11, P > 0.096 and MS = 0.01, DF = 1, F = 0.17, P > 0.678 for nitrogen and previous damage treatments, respectively. For larvae: MS = 45.51, DF = 3, F = 1.39, P > 0.243 and MS = 88.51, DF = 1, F = 2.71, P > 0.099 for nitrogen and damage treatments, respectively).

The mean number of aphids found on plants was influenced by the sampling date, previous plant damage and the amount of nitrogen applied (Table 3). Significantly more aphids were found on plants in the first than the second sampling date for all nitrogen levels and all plants previously damaged or undamaged (Fig. 4A). Previous plant damage and nitrogen applied had no significant effect on the number of aphids present in the first sampling date, but nitrogen application levels affected aphid numbers on the second sampling date which accounts for the date x nitrogen x damage interaction (Table 3, Fig. 4A & 4B). Pooling the number of aphids with respect to plant damage indicates that date and nitrogen have a strong effect on the number of aphids present (Fig. 4B, MS = 94.87, DF = 1, F = 332.54, P < 0.001 and MS = 2.29. DF = 3, F = 8.03, P < 0.001 for date and nitrogen treatments, respectively). Significantly more aphids were found on the first than the second sampling date (Fig. 4B). There was a non significant trend of increased numbers of aphids with nitrogen on the first sampling date with the highest numbers within plots with 60 lb/acre nitrogen (Fig. 4B).

The number of fire ants was significantly influenced by the amount of nitrogen applied and previous plant damage (Table 4). Although no significant differences were found among mean numbers of ants within all plots and plant damage and nitrogen, a trend showing increasing numbers of ants with increasing nitrogen level on undamaged plants and increasing numbers of ants on damaged plants in low nitrogen plots was apparent (Fig. 4C). Significantly more ants were found on the first than the second sampling date (Fig. 4D).

The mean number of lacewing eggs was significantly influenced by date, nitrogen and previous plant damage (Table 4). Significantly fewer lacewing eggs were found on the first than the second sampling date for all nitrogen applications and all plants previously damaged or undamaged (Fig. 5A). On the second sampling date, significantly higher numbers of lacewing eggs were found on previously damaged plants in plots with higher nitrogen (Fig 5A). Pooling plots with 0 & 30 and 60 &120 lb/acre nitrogen applied on the second sampling date show a significant influence of previously damaged plants with higher nitrogen on the mean number of lacewing eggs on plants (Fig. 5B, MS = 161.03, DF = 1, F = 6.02, $P < 0.016$ for the interaction of nitrogen & plant damage).

Sampling date and previous plant damage significantly influenced the number of lacewing larvae and pupae (Table 4). Significantly fewer lacewing larvae and pupae were found on the previously damaged than undamaged plants on the first sampling date although the number of eggs were the same (Fig. 5C & 5D). There were no differences in the mean number of lacewing larvae and pupae on plants on the second sampling date, but the trend follows the number of their eggs found on this date (Figure 5C & 5D).

The number of coccinellid adults was significantly influenced by the date of sampling (MS = 5.43, DF = 1, F = 17.61, $P < 0.001$). Significantly more adults were found on the first than the second sampling date (Mean number of adults per plant = 0.35 ± 0.66 (SD) & 0.18± 0.44 for first and second dates, respectively. N = 320 plants/date). The number of coccinellid eggs, larvae and pupae was also significantly influenced by sampling date (MS = 183.83, DF = 1, F = 13.31, $P < 0.001$). Significantly more coccienellid eggs, larvae and pupae were found on the first than the second sampling date (Mean number of eggs, larvae and pupae per plant = 1.55 ± 4.55 (SD) & 0.48 ± 2.67 for the first and second sampling date, respectively. N = 320 plants/date). There were no significant effects from the nitrogen and previous plant damage treatments or their interactions on the number of coccinellid adults or eggs, larvae and pupae.

The number of spiders was marginally influenced by the sampling date (MS = 2.63, DF = 1, F = 3.71, P = 0.055). Fewer spiders were found on the first than the second sampling date (Mean number of spiders per plant = 0.38 " 0.72 (SD) & 0.51 " 0.98 for the first and second sampling dates, respectively, N = 320 plants/date). There were no significant effects from the nitrogen and previous plant damage treatments or their interactions on the number of spiders present.

There were no significant effects from date, nitrogen and previous plant damage or their interactions on the number of adult parasitoids present.

DISCUSSION

There was a general pattern of increasing numbers of *H. zea* and *H. virescens* eggs with increasing nitrogen. In addition, previous plant damage had a significant effect on the number of eggs found only at the higher nitrogen levels. As a result of these ovipositions, the larvae of these species also follow this general trend. It is not clear what the mechanism(s) is that allows for increased presence of these species on damaged plants in high nitrogen plots. Predation/parasitism of eggs and larvae may be lower on high nitrogen plants that had been previously damaged, and/or moths may be responding to differences in the chemical/visual properties of high nitrogen plants that had been previously damaged. Plants were taller in the highest nitrogen plots and previous reports indicate that several lepidopteran species prefer to lay their eggs on taller plants with high nitrogen (Hern et al. 1996). We did not assess predation/parasitism of eggs and larvae in this study and the eggs had not hatched at the final sampling and prior to harvest. Further investigations of *H. zea* and *H. virescens* responses to higher nitrogen and previously damaged plants and the effect on their survival will be the subject of subsequent studies.

Aphids increase in numbers with nitrogen but at the highest nitrogen levels they begin to decline producing a dome shaped distribution across nitrogen amounts. The distribution of fire ants closely followed that of aphids. It may be that aphids respond to nitrogen in a linear manner and that the population on the highest nitrogen plots began to crash at an earlier date.

Total fruit production and damage was highest in the plots with the highest nitrogen, but neither fruit production nor damage was influenced by previous plant damage by *S. exigua*. The yield across all nitrogen levels, even in the plots where no nitrogen was applied (crimson clover only) were not significantly different.

Lacewing eggs follow the same pattern as *H. zea* and *H. virescens* eggs. More lacewing eggs were found on higher nitrogen plants that had been previously damaged. The number of larvae and pupae of these species follow this trend only on the second sampling date. Very few lacewing larvae or pupae were found throughout the season compared to the number of eggs that were found. Lacewing eggs hatch in 3-4 days, which suggests high larval and pupal predation early in the season. The lacewing eggs counted had not hatched at the time of sampling. Therefore, further investigations of lacewing responses to higher nitrogen and previously damaged plants and the effect on their survival will be the subject of subsequent studies.

There was a strong interaction between nitrogen, previous plant damage and the insect species present with a general pattern of increased fruit damage on higher nitrogen plants. Based on an earlier study showing that plants could improve their fitness through previous damage by attracting parasitoids of the pest species, we would expect to find decreased oviposition on previously damaged plants. We found higher oviposition in the case of *H. zea* and *H. virescens* and lacewings. However, this preference was more the case with high nitrogen, thus indicating that the nature of plant signals may have been altered by nitrogen rates in such a manner that the pest perceives a weakened plant and the predator perceives higher numbers of prey.

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Table 1. Anova Testing the Effects of Date, Replication Nested Within Date, Nitrogen Level, and the Interaction Between Date and Nitrogen on the Total Number of Squares and Bolls Produced (Total Fruit), the Number of Damaged Squares, the Number of Damaged Bolls, and the Percent Damage of Squares and Bolls after Arcsine Square Root Transformation. N = 640 Cotton Plants.

Factor	DF	MS	F
Total Fruit			
Date	1	60314.64	454.16***
Replication (Date)	6	342.32	2.58*
Nitrogen	3	496.94	3.74**
Date x Nitrogen	3	50.26	0.38
Damaged Squares			
Date	1	1.81	0.57
Replication (Date)	6	46.67	14.75***
Nitrogen	3	17.42	5.50**
Date x Nitrogen	3	6.85	2.17
Damaged Bolls			
Date	1	124.26	135.29***
Replication (Date)	6	2.00	2.18*

Nitrogen	3	6.47	7.05***
Date x Nitrogen	3	6.77	7.37***
% Total Fruit Damaged			
Date	1	0.09	2.23
Rep (Date)	6	0.40	10.05***
Nitrogen	3	0.26	6.59***
Date x Nitrogen	3	0.21	5.16**
Plant Height			
Date	1	34061.81	1737.72***
Rep (Date)	6	1216.38	62.06***
Nitrogen	3	264.61	13.50***
Date x Nitrogen	3	72.39	3.69*

Significant at *0.05, **0.01, ***0.001

Table 2. Anova Testing the Effects of Replication, Nitrogen Level, Beet Armyworm Damage, and Their Interaction on the Total Number of Squares and Bolls Produced (Total Fruit), the Number of Damaged Squares, the Number of Damaged Bolls, and the Percent Damage of Squares and Bolls after Arcsine Square Root Transformation. N = 320 Cotton Plants.

Factor	DF	MS	F
Total Fruit			
Rep	3	308.50	1.57
Dud	1	252.05	1.28
Nitrogen	3	220.62	1.12
Damage x Nitrogen	3	315.29	1.60
Damaged Squares			
Rep	3	59.71	19.61***
Dud	1	0.61	0.20
Nitrogen	3	13.66	4.49**
Damage x Nitrogen	3	0.67	0.22
Damaged Bolls			
Rep	3	3.98	2.17
Dud	1	1.01	0.55
Nitrogen	3	13.23	7.22***
Damage x Nitrogen	3	0.64	0.35
% Total Fruit Damaged			
Rep	3	0.38	18.59***
Dud	1	0.02	0.93
Nitrogen	3	0.23	11.35***
Damage x Nitrogen	3	0.01	0.39

Significant at *0.05, **0.01, ***0.001

Table 3. Anova Testing the Effects of Date, Replication, Nitrogen Level, Beet Armyworm Damage and Their Interactions on Log-transformed Number of *H. Zea* & *H. Virescens* Eggs, *H. Zea* & *H. Virescens* Larvae & Pupae, Aphids and Fire Ants. Type Iii Sums of Squares for Error. N = 640 Cotton Plants.

Factor	DF	MS	F
H. zea, H. virescens eggs			
Date	1	1.52	12.97***
Replication (Date)	6	2.83	24.13***
Nitrogen	3	0.22	1.84
Damage	1	0.68	5.80*
Date x Nitrogen	3	0.10	0.88
Date x Damage	3	0.41	3.53*
Nitrogen x Damage	1	0.74	6.32*
Date x Nitrogen x Damage	3	0.16	1.38
H. zea, H. virescens larvae			
Date	1	10.00	30.19***
Replication (Date)	6	2.23	6.73***
Nitrogen	3	1.10	3.33*
Damage	1	2.50	7.55**
Date x Nitrogen	3	0.62	1.85
Date x Damage	3	0.35	1.07

Nitrogen x Damage	1	2.03	6.11*
Date x Nitrogen x Damage	3	0.18	0.54
Aphids			
Date	1	94.87	336.50***
Replication (Date)	6	53.81	190.85***
Nitrogen	3	2.29	8.13***
Damage	1	0.26	0.92
Date x Nitrogen	3	0.33	1.18
Date x Damage	3	1.01	3.57*
Nitrogen x Damage	1	0.15	0.52
Date x Nitrogen x Damage	3	0.98	3.49*

Significant at *0.05, *0.01, ***0.001

Table 4. Anova Testing the Effects of Date, Replication, Nitrogen Level, Beet Armyworm Damage and Their Interactions on the Number of Lacewing Eggs, Lacewing Larvae & Pupae and Fire Ants. Type Iii Sums of Squares for Error. N = 640 Cotton Plants.

Factor	DF	MS	F
Lacewing eggs			
Date	1	2476.7	136.61***
Replication (Date)	6	275.60	15.20***
Nitrogen	3	103.59	5.71***
Damage	1	169.13	9.33**
Date x Nitrogen	3	23.19	1.28
Date x Damage	3	9.64	0.53
Nitrogen x Damage	1	161.00	8.88**
Date x Nitrogen x Damage	3	56.26	3.10*
Lacewing larvae & pupae			
Date	1	0.08	1.06
Replication (Date)	6	0.12	1.72
Nitrogen	3	0.07	1.00
Damage	1	0.13	1.75
Date x Nitrogen	3	0.04	0.60
Date x Damage	3	0.56	7.78**
Nitrogen x Damage	1	0.05	0.71
Date x Nitrogen x Damage	3	0.02	0.31
Fire ants			
Date	1	153.08	4.20*
Replication (Date)	6	216.00	5.92***
Nitrogen	3	36.09	0.99
Damage	1	7.01	0.19
Date x Nitrogen	3	100.57	2.76*
Date x Damage	3	29.21	0.80
Nitrogen x Damage	1	3.16	0.09
Date x Nitrogen x Damage	3	36.73	1.01

Significant at *0.05, **0.01, ***0.001

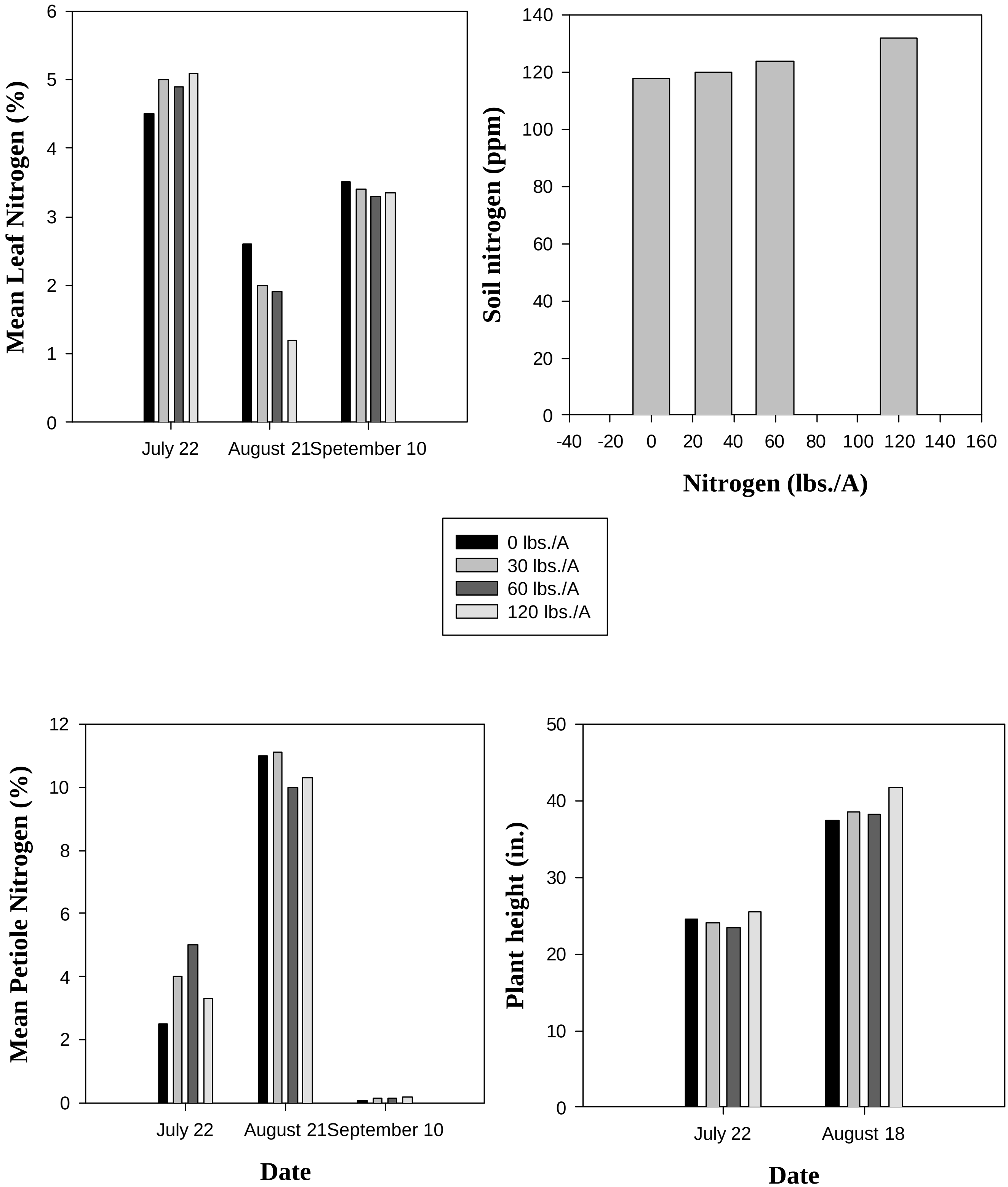


Figure 1. Mean percentage of leaf (A) and petiole (B) nitrogen across sampling dates. Mean parts per million (ppm) of soil nitrogen (C), and mean plant height (D). Samples were taken from 4 plots with 0, 30 60 & 120 lb/acre nitrogen applied across 4 replications, *n* = 16

plots (C). Treatments with different letters are significantly different at *p* < 0.05.

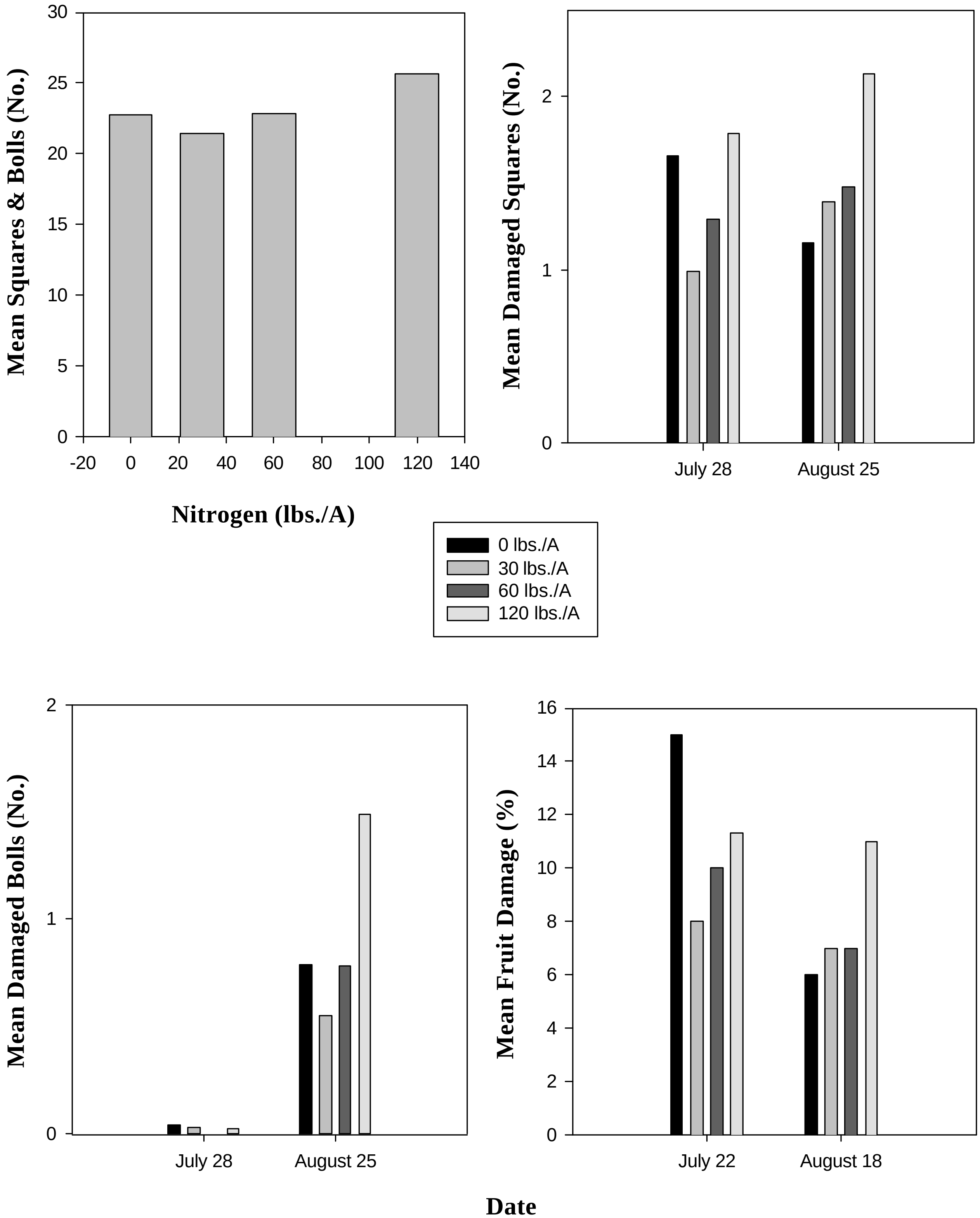


Figure 2. Mean number of squares and bolls per nitrogen applied to plots (A), mean number of damaged squares (B) and damaged bolls (C) per sampling date and nitrogen applied to plots, and mean percent of square and boll damage per sampling date and nitrogen applied to plots (D), *n* = 640 plants. Treatments with different letters are significantly different at *p* < 0.05.

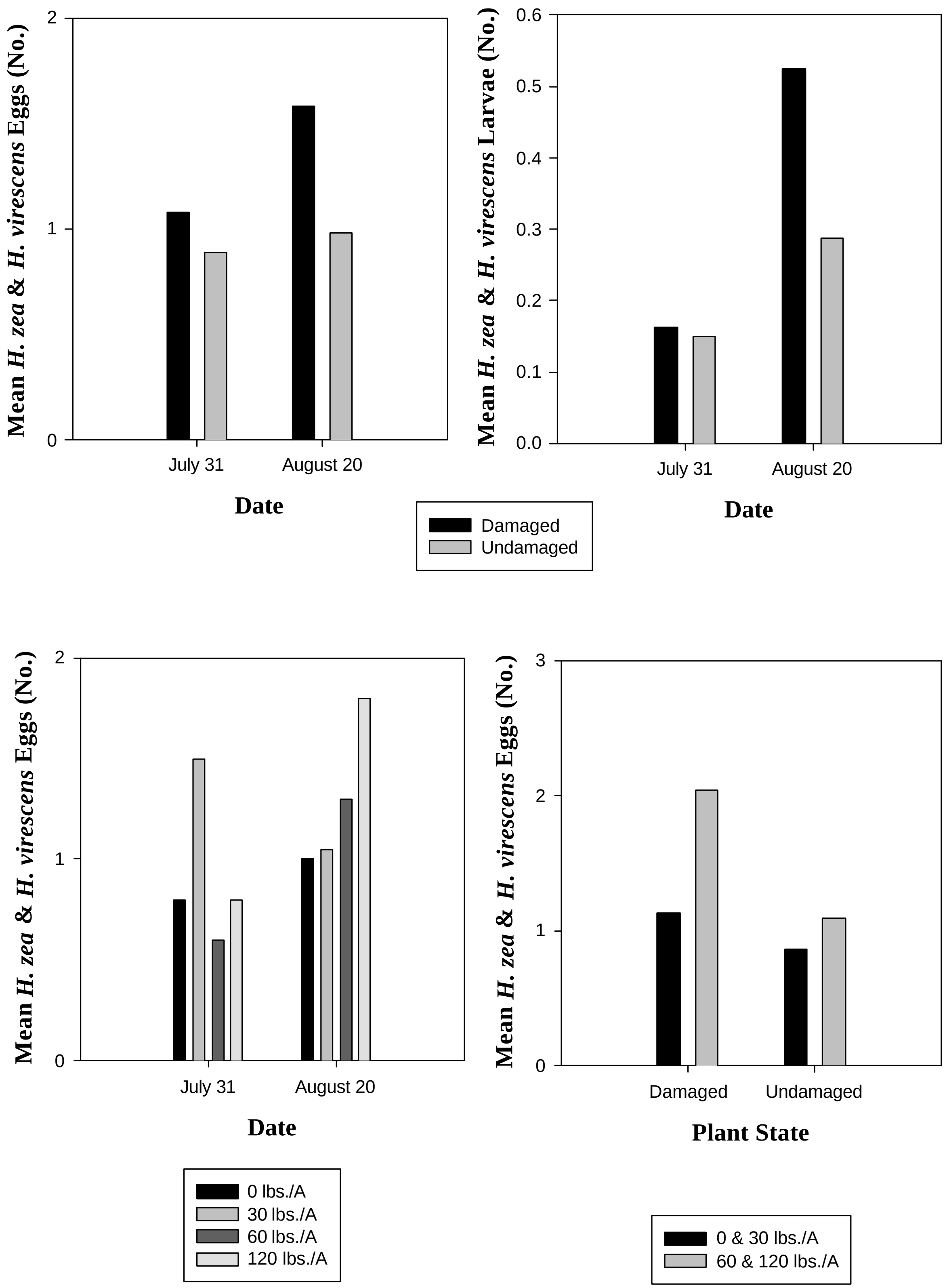
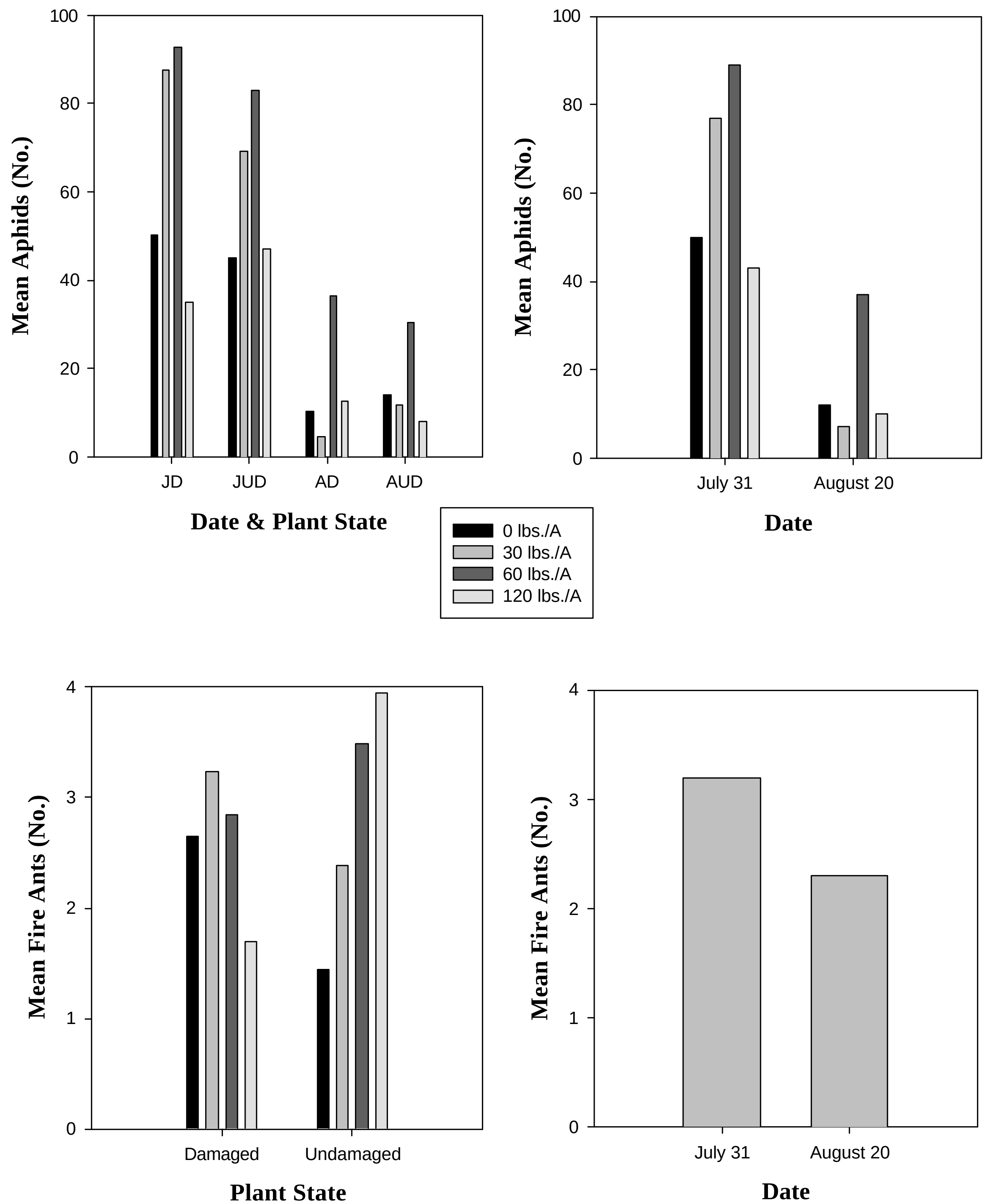


Figure 3. Mean number of *Helicoverpa zea* and *Heliothis virescens* eggs (A) and larvae (B) per sampling date on previously damaged and not previously damaged plants, and per nitrogen applied to plots (C), *n* = 640 plants. Mean number of *H. zea* and *H. virescens* eggs on August 20 for previously damaged and not previously damaged cotton plants after pooling nitrogen into 0& 30 and 60 & 120 lb/acre nitrogen applied to plots (C), *n* = 320 plants. Treatments with different letters are significantly



different at $p < 0.05$. Figure. 4. Mean number of aphids on July (J) and August (A) per previously damaged (D) and not previously damaged (UD) cotton plants across nitrogen applied to plots (A), and across sampling dates and nitrogen applied to cotton plots (B). Mean number of fire ants per previously damaged and not previously damaged plants across nitrogen applied to plots (C) and across sampling dates (D). N = 640 plants. Treatments with different letters (nested within date in Fig.4A)

are significantly different at p < 0.05.

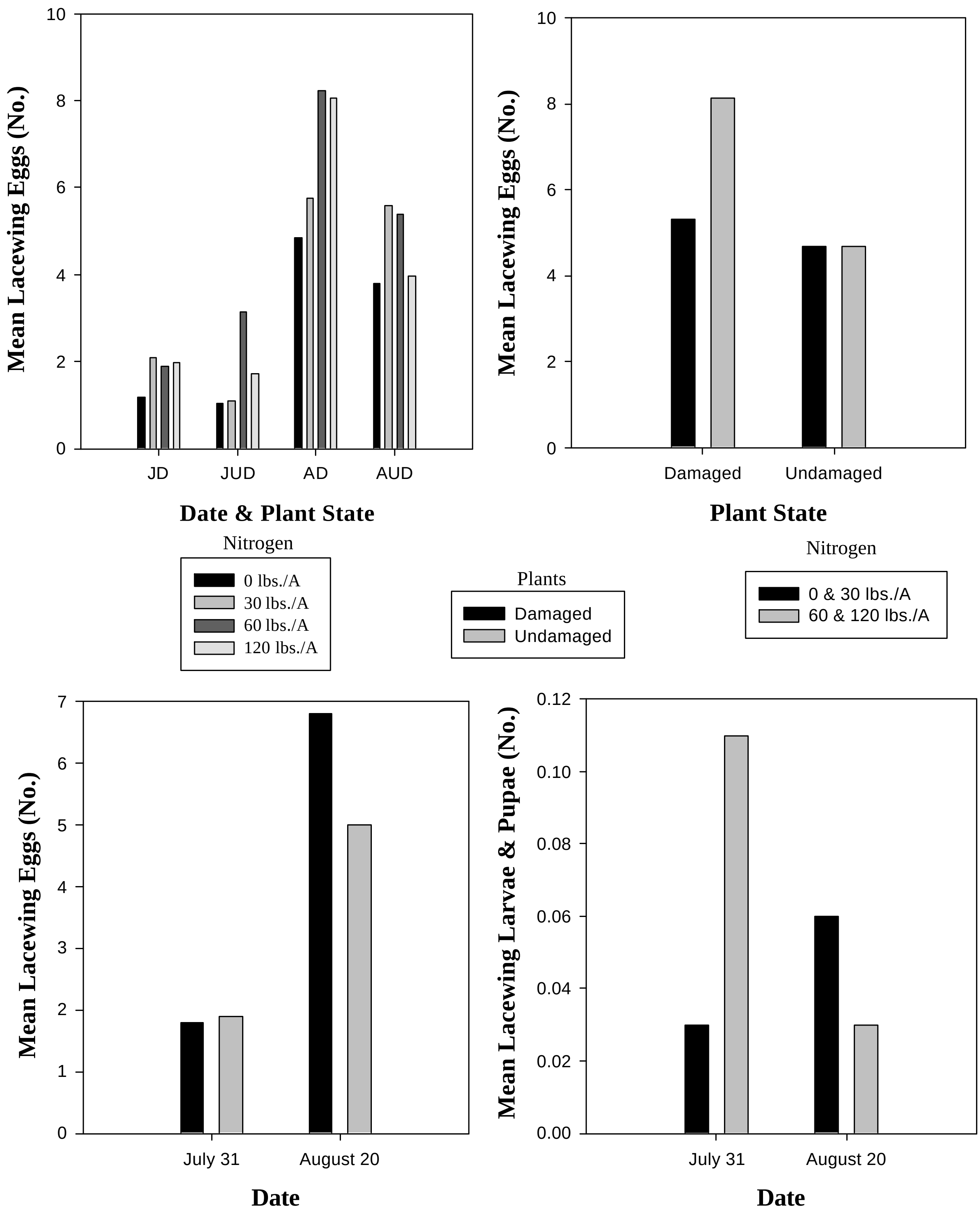


Figure. 5. Mean number of lacewing eggs on July (J) and August (A) per previously damaged (D) and not previously damaged (UD) cotton plants across nitrogen applied to plots (A), *n* = 640 plants. Mean number of lacewing eggs on August 20 for previously damaged and not previously damaged cotton plants after pooling nitrogen into 0 & 30 and 60 & 120 lb/acre nitrogen applied to plots (B), *n* = 320 plants. Mean number of lacewing eggs (C) and larvae & pupae (D) across previously damaged and undamaged plants and sampling dates, *n* = 640 plants. Treatments with different letters (nested within date in fig. 5A) are significantly different at *p*

< 0.05.