

Influences of Soil Fertility on Spatial Patterns of *Aphis Gossypii* Glover (Homoptera: Aphididae) Occurred in Bt-cotton Plants

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Abstract: With the widespread planting of bacterium *Bacillus thuringiensis* (Bt)-cotton, non-target piercing-sucking insects such as *Aphis gossypii* Glover (Homoptera: Aphididae) have become the main hazard of cotton. Fertilization influences the distribution and population dynamics of aphid seriously. In this study, the effects of Nitrogen (N) and Phosphorus (P) on aphid population density were investigated on Bt-cotton; the combinational impacts of nitrogen and potassium (K) fertilizers on the distribution of aphid were examined as to guide rational fertilization to reduce pests and environmental pollution. Aphid density in cotton plants fertilized with 72 kg/ha N (84.2 ± 22.7 aphids/plant) was significantly higher than fertilized with 0 and 108 kg/ha N (36.7 ± 4.0 and 47.8 ± 18.7 aphids/plant). Compared to cotton plants treated with 23 and 69 kg/ha P, aphid density was higher (61.67 ± 21.08 aphids/plant) in cotton plants treated with 46 kg/ha P. Comparison of spatial distribution of aphid population showed that aphid preferred to suck the sap of bottom fruit branches in cotton plants treated by single K fertilizer and combinational fertilizers of N and K. Thus, these results suggested that proper application of fertilizers should be beneficial to controlling phytophagous insects in Bt-cotton production.

Keywords: Aphid, bt-cotton, distribution, fertilization, population dynamic

INTRODUCTION

Fertilization is an important procedure in crop management and plays key role in increasing crop yield. Some macroelements such as Nitrogen (N), Phosphorus (P) and potassium (K) were extensively applied to promote crop production. More and more consequences revealed that a lot of nitrate fertilizers applied by farmers not only increased nitrate and soluble acids content in plants (Mengel and Kirkby, 2001), but also improved the nutritional quality and infestation of phytophagous insects (Van Emden, 1966; Minkenberg and Fredrix, 1989; Bentz *et al.*, 1995).

Cotton is one of the main economic crops in China, *Helicoverpa armigera* and cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae) are the most serious pests. Aphid has been becoming the most common leaf-chewing herbivore infesting cotton with the widespread planting of the bacterium *Bacillus thuringiensis* (Bt)-cotton. Aphid feed from phloem on the undersides of cotton leaves by stylet. Damages to cotton resulted from leaf crinkling, accumulations of honeydew and associated problems of sooty mold (Akey and Butler, 1989). Honeydew contamination causes problems in cotton lint processing at the gin and textile mill (Slosser *et al.*, 1989; Ebert and Cartwright, 1997).

Variety of pesticides was applied in the field management. However, chemical control may require scouting, kill beneficial insects and cause environmental pollution (Zhang *et al.*, 2010). Despite substantial assistance from beneficial organisms, cotton aphid populations were often widespread and persistent, resulting in some cases of maturity delays and possible yield losses in a number of cotton fields. Southeast Farm Press reported that, prior to 1989, low numbers of cotton aphids were the rule in North Carolina and could be routinely controlled by any of a number of Organophosphate (OP) insecticides (Bachelier, 2003). As had happened earlier in the Mid-South, by 1990 some aphid population in North Carolina appeared to be expressing high levels of resistance to recommended OP insecticides (Gubran *et al.*, 1993; Kerns and Gaylor, 1993).

Compared to chemical control, biological control was considered to be a long term, sustainable means for pest management. Studies have identified several native or exotic natural enemies of aphid (Fox *et al.*, 2004; Heimpel *et al.*, 2004; Desneux *et al.*, 2006). However, most natural enemies can only control aphids when they are at a low density. When the aphid density reaches an economic threshold, insecticides application might be required to avoid economic loss.

In order to reducing economic losses, cutting down money and manpower input and lessening non-point source pollution, we attempted to find the relationship between fertilization and aphid dynamics. In this study, different levels of N, P and K fertilizer treatments were designed; aphid density in different treatments and within-plant distribution was investigated as to provide fertilization formulation to reduce aphid damage.

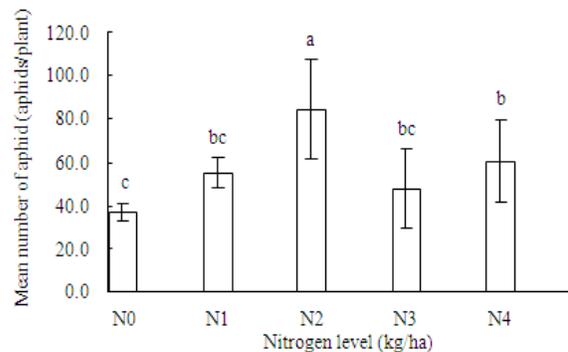
MATERIALS AND METHODS

Experiment site and cotton: Experiment site was selected at the station of Yangtze University, Hubei province, Yangtze River basin, one of the three biggest cotton production areas of China. Sanza cotton (which has Cry1Ac gene), bred by Hubei Academy of Agricultural Sciences were chosen to be the experimental seeds. Bt-cotton seeds were sowed in nutritional plots in greenhouse of Yangtze University experimental base on 25 March, 2010 and transplanted into experimental field when cotton seedlings with three to four leaves on 15 April. The seedlings were distributed in different 20 m² plots with 2 rows of protection cotton plants. In each row, cotton plants were planted in two lines with 30 cm-space; 40 cm was designed between each plant in each line (Fig. 1). All experimental cotton managed with conventional style then infested by aphid and no chemical pesticide was used during the field trials (Ai *et al.*, 2010).

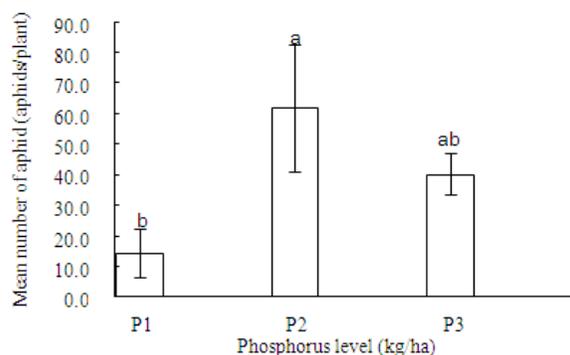
Different letter (s) above the bars indicated significant differences of aphid density between fertilization levels, pooled across sampling dates, at $p \leq 0.05$ as determined by repeated measures one-way ANOVA, based on untransformed mean values and followed by the Fisher's protected Least-Significant Difference (LSD) test. Figure 1(a) presents the mean values of aphids in cotton plant under 5 nitrogen levels (one-way ANOVA for aphids number: $F_{4, 8} = 8.807$, $p < 0.001$). Figure 1(b) presents the average number of aphids under 3 phosphorus levels and each level was combined with N2K2 (one-way ANOVA for aphids density: $F_{2, 4} = 9.995$, $p = 0.028$).

Fertilization design: Five nitrogen levels combined with 3 potassium levels and 3 phosphorus levels according to the conventional fertilization of cotton in central China was designed (Table 1). Fertilizers combined with different ratios of N, P and K was applied in experimental field based on fertility test results. P and K was applied as base fertilizer one-time, 60% ammonium bicarbonate was applied as base fertilizer and the remaining 40% was applied as topdressing (10% of which was applied during transplanting period, squaring stage, initial flowering period and full-bloom stage respectively).

Sampling method: Cotton plants in fertilizer treatments and control plots were invaded by aphid naturally in the wild. Observation time was arranged on 15 July, when aphid population displayed the main



(a)



(b)

Fig. 1: Effect of nitrogen and phosphorus fertilizers on population dynamics of *Aphis gossypii* glover separately

Table 1: Different levels of Nitrogen (N), Phosphorus (P) and potassium (K) fertilizer designed in the study

Item	level 1	level 2	level 3	level 4	level 5
N	0	36	72	108	144
P	23	46	69	-	-
K	60	120	180	-	-

N: Ammonium carbonate; P: Single superphosphate; K: KCl
Numbers in each column represent the amount of fertilization application (kg/ha)

peak time. All leaves on 1-11th fruit branches of 10 randomly selected cotton plants in each plot were checked at 5-7 days interval. Aphid population in 9 repetitions of every treatment was counted except for the alate adults. Observation was postponed on rainy day.

Statistical analysis: Sampling data of aphid population in treatments managed by independent effect of N and P was transferred into the mean number of aphid on per plant. Aphid density in treatments managed by combinational effects of N and K was transferred into the number of aphid on per fruit branch. Results of N and P effect on aphid density was calculated and compared using one-way Analysis of Variance (ANOVA) and two-way ANOVA was used to evaluate the interactive effect of N and K fertilizer on aphid

Table 2: Effect of fertilization levels on spatial distribution of aphid population in cotton plants

Fertilization level ^a	Aphid population in plant strata ^b			Fertilization effect (mean±S.D.)
	Aphid density in basal strata (mean values) ^c	Aphid density in middle strata (mean values) ^c	Aphid density in apical strata (mean values) ^c	
N0P1K1	48.29±10.87 a, A	28.73±5.00 ab, AB	10.35±4.90 b, B	29.12±27.69 a
N0P1K2	19.48±5.77 a, A	18.74±8.00 a, A	7.07±2.24 a, A	15.10±18.72 ab
N0P1K3	28.14±8.00 a, A	17.43±6.27 ab, A	4.71±2.80 b, A	16.76±20.96 ab
N1P1K1	62.40±24.00 a, A	24.29±3.05 b, B	2.56±1.26 c, B	29.75±49.55 bc
N1P1K2	19.03±4.88 a, A	18.35±3.44 a, A	11.94±6.61 a, A	16.44±16.02 bc
N1P1K3	33.16±6.51 a, A	26.67±5.49 a, AB	5.04±2.20 b, B	21.62±19.74 bc
N2P1K1	23.37±4.44 a, A	17.31±5.65 a, A	8.08±3.92 a, A	16.25±15.77 bc
N2P1K2	28.94±6.89 a, A	19.27±7.48 ab, A	2.91±1.61 b, A	17.04±21.17 c
N2P1K3	25.14±6.65 a, A	32.70±7.80 ab, A	9.22±4.72 b, A	22.35±22.23 c
N3P1K1	21.89±5.24 a, A	14.35±4.27 a, A	3.06±1.92 a, A	13.04±14.67 c
N3P1K2	26.83±6.78 a, A	28.82±8.32 a, A	9.56±3.61 a, A	21.74±21.79 c
N3P1K3	28.50±10.84 a, A	21.28±5.48 ab, A	3.35±1.34 b, A	17.71±24.07 c
N4P1K1	37.84±11.03 a, A	15.85±7.30 b, AB	6.13±2.61 b, B	19.94±27.32 c
N4P1K2	21.55±7.20 a, A	14.51±3.44 a, A	4.74±1.74 a, A	13.60±16.00 c
N4P1K3	84.57±14.52 a, A	26.42±4.08 b, B	3.71±1.14 c, B	38.23±43.70 c
Plant strata effect (mean±S.D.)	33.94±35.29 a, A	21.65±18.72 b, B	6.16±10.16 c, C	

^a: Fertilization levels: N0 = 0 kg/ha, N1 = 36 kg/ha, N2 = 72 kg/ha, N3 = 108 kg/ha, N4 = 144 kg/ha, P1 = 23 kg/ha, K1 = 60 kg/ha, K2 = 120 kg/ha, K3 = 180 kg/ha; ^b: Differences between mean values within row sharing the same capital letter (s) are not significantly different ($p \geq 0.05$) as determined by two-way Analysis of Variance (ANOVA) and followed by the Fisher's protected Least-Significant Difference (LSD) method after arcsine transformation (two-way ANOVA for aphid population: $F = 58.696$, $p < 0.001$, fertilization levels: $F = 2.944$, $p < 0.001$, and interactions between fertilization levels and aphid population in plant strata: $F = 2.321$, $p < 0.001$); ^c: Basal strata: 1-3 fruit branches; middle strata: 4-7 fruit branches; apical strata: 8-11 fruit branches, each mean is based on a sample size of 9

within-plant distribution followed by the Fisher's protected Least-Significant Difference (LSD) method. Statistical analyses were performed using DPS program (v7.05). A probability level of $p < 0.05$ was considered as statistically significant.

RESULTS

Effect of different N and P levels on the density of aphid: N effect on aphid population was observed based on the same level of P and K. Aphid number on the N level of 72.0 kg/ha (84.23±22.70) has very significant difference compared with the other treatments, five series meet the normal distribution basically (Fig. 1).

Meanwhile, P effect on aphid population based on unchanged application amount of N and K fertilizers was observed. The mean number of aphid in different treatments fertilized with P1, P2 and P3 also consistent with the normal distribution. There was very significant difference between treatments fertilized with 46 and 23 kg/ha P (61.67±21.08 and 14.11±7.86, respectively), significant difference presented between 46 and 69 kg/ha P (40.11±6.88).

Combinational effect of N and K on within-plant distribution of aphid: Our study showed that the combinational fertilization of N and K in different rate did influence the within-plant distribution of aphid. Aphid densities in basal strata (28.94±6.89) were significantly higher than that in apical strata (2.91±1.61) in N2P1K2 treatment; aphid densities in basal strata (25.14±6.65) were significantly higher than that in apical strata (9.22±4.72) in N2P1K3 treatment;

aphid densities in basal strata (28.50±10.84) were significantly higher than that in apical strata (3.35±1.34) in N3P1K3 treatment; aphid densities in basal strata (37.84±11.03) were very significantly higher than that in apical strata (6.13±2.61) in N4P1K1 treatment; aphid densities in basal strata (84.57±14.52) were very significantly higher than that in apical strata (3.71±1.14) in N4P1K3 treatment (Table 2). More cotton aphid preferred the basal fruit branches to the apical ones with the increasing K fertilizers, but this trend was not obvious in treatments fertilized with different K levels combined with N0 and N1.

Our experiment demonstrated that the aphid population in Bt-cotton fertilized with N levels from low to high meet normal distribution, but there were negative correlation between aphid densities and K levels generally. That is, the impact on aphid within-plant distribution of N levels beyond N2 was similar to the K levels beyond K2. Consequently, more aphids preferred to the basal fruit branches with the increasing K fertilization and this trend was more obvious in plots with combinational fertilization of N2, N3 and N4.

DISCUSSION

Cotton plant formed morphological resistance to aphid by specificity of organ or tissue, such as inserted position, size, strength, color and structure (Guo *et al.*, 1993; Xia *et al.*, 1996). Moreover, cotton against aphid invasions by biochemical resistance in the following three ways:

- **First, avoid and inhibit action:** Such as terpenoid compounds, hinder the feeding of aphid and keep them away (Sachs, 1996; Garry, 1999).

- **Second, the impact to aphid digestion:** Tannin, lignin, soluble sugar and amino acid for instance, negative to aphid digestion seriously and cause dyspepsia (Wang *et al.*, 1987; Odani and Ikenaka, 1976).
- **Third toxic action:** Gossypol, quercetin, enzymes and so on, restrain the growth and development of aphid, reduce the rate of reproduction or lead to poisoned (Gary *et al.*, 1976; Bell and Stipanovic, 1978; Stipanovic, 1988).

Fertilization influenced the dynamics of aphid population by changing the morpha of cotton tissue and the synthesis of insect-resistant secondary compounds.

Nitrogen is one of many important components of organic compounds, just as proteins, nucleic acids, chlorophyll, enzymes and alkaloids. Appropriate increase of nitrogen fertilizer made stem and leaf tissue much softer, more fresh and crisp, improved the nutritional quality of plants and infestation of aphid. Majority of nitrogen concentrated in young organs in the vegetative growth phase, old growth stems and leaves is low, that is why more aphids preferred apical and middle stratum to basal ones. Previous studies indicated that adequate leaf nitrogen enhanced both growth and reproduction of herbivorous insects (Dixon, 1997; Mattson, 1980; Larsson, 1989; Waring and Cobb, 1992; Douglas, 1993; Slosser *et al.*, 1998) and reduced the susceptibility herbivore insects to some insecticides (McKenzie *et al.*, 1995). But nitrogen also plays an important role in the synthesis of sugar which can disturb the digestive system of aphid, so excessive nitrogen application decreased the edibility of aphid.

Phosphorus can improve the hydrature of protoplasm colloid and saturation of cell structure, maintain colloid state and increase the viscosity and elasticity of protoplasm, conducive to herbivores feeding. Organic compounds containing phosphorus bridged by phosphate, such as nucleic Acids, Phospholipids, nucleotides, Triphosadenine (ATP), etc., play an extremely important part in plant metabolism. Phosphotriose (TP) formed in chloroplast under photosynthesis exchanged with phosphate in cytoplasm mostly when phosphorus application was adequate, TP was transferred to cytosol and conveyed to the organic centre in time through a series of transformation process. Carbohydrate metabolism will be suffocated with deficient phosphorus, cause accumulation of sugar and disturb aphid feeding. On the contrary, excessive phosphorus will strengthen the respiration of plant; consume a lot of sugar and energy (Lu and Zhang, 2003).

Adequate supply of potassium could thicken the cell wall, advanced cell lignifications, enhanced the osmotic potential of cells and increased the turgor, thereby prevented or reduced pathogen invasion and insect damage. Fuchs and Grossmann demonstrated that the resistance of crops depended on the ratio of nitrogen

and potassium greatly: when potassium level was low, protein synthesis in plant tissue would be reduced, even be disintegrated that might result in amine poisoning (mainly in old leaves). In addition, potassium acted on nitrogen metabolism and aphid population dynamics by activating enzyme, for example, 6-phosphofructokinase, glucose-starch synthase, ADP (UDP) glucose pyrophosphorylase, ATP phosphohydrolase (more than 60 enzymes) and so on. Other report published that, cotton with proper amount of potassium can accumulate phytoalexins, phenols and auxin around the sick point of plant, prevent the expansion of pests and disease and benefit to callus forming (Cotton Research Institute, CAAS, 2003).

Because agro-ecosystem is an open system, which is affected by a variety of ecological factors, such as wind directions, light and precipitations, so the aphid population dynamics and within-plant distribution evaluated above may be the result of comprehensive functions of various factors. Crop yield largely depends upon synthetic fertilizers input in modern agriculture, excessive fertilization waste a large amount of resources, reduce fertilizer utilization and led to the formation of non-point source pollution in the meantime (Ge, 2003; Li *et al.*, 2008; Peng *et al.*, 2001). A better understanding of rational fertilization is not only concentrated on increasing production, but also on the sustainable development of agriculture.

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