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Full Length Research Paper

The soybean aphid, *Aphis glycines* (Hemiptera: Aphididae): Population dynamics on edamame soybeans in Nebraska, USA

Bamphitlhi Tiroesele[1]*, Thomas E. Hunt[3], Robert Wright[2], Erin E. Blankenship[4] and John E. Foster[2]

[1]Botswana College of Agriculture, Private Bag 27, Gaborone, Botswana.
[2]Department of Entomology, University of Nebraska-Lincoln, Lincoln, NE 68583-0816, USA.
[3]Department of Entomology, University of Nebraska Haskell Agricultural Laboratory, Concord, NE, 68728-2828, USA.
[4]Department of Statistics, University of Nebraska-Lincoln, Lincoln, NE 68583-0963, USA.

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Edamame are soybeans harvested at a physiologically immature (R6) stage as a specialty food item for fresh and processed (frozen) markets. The soybean aphid, *Aphis glycines* Matsumura, is a newly introduced insect pest of soybeans in North America. This field study was to provide baseline information on the impact of *A. glycines* on the edamame soybean. This study determined the population growth rate of *A. glycines* on two edamame soybean cultivars, 'Butterbeans' and 'Envy,' at two planting dates during 2004 and 2005 in Nebraska. Aphid population growth was significantly higher on 'Butterbeans' than on 'Envy' for the first planting date in both 2004 and 2005 seasons, whereas the second planting date only had significant higher soybean infestations on 'Butterbeans' during the 2005 season. The infestation difference was the greatest on plants at the late reproductive growth stages, R5 and R6, in 2005. Aphid's infestation at 'Butterbeans' growth stages in 2005 was significantly different for the first and second planting dates. The aphids were higher on plants at the R6 and R5 growth stages than the other stages for first and second planting dates, respectively. However, 'Envy' growth stages in 2005 did not exhibit significantly different average aphid infestation during the first and second planting dates. This study revealed that soybean aphid population growth on edamame soybeans is affected by the planting date, season, and cultivar choice.

Key words: Growth stages, cultivars, infestation, planting date.

INTRODUCTION

Green vegetable soybean or edamame is a specialty soybean (*Glycine max* L. Merrill) harvested as a vegetable when the seeds are immature. Edamame is consumed mainly as a snack, but also as a vegetable, an addition to soups, or processed into sweets. The crop is gaining popularity throughout Asia and the United States (Sirisomboon et al., 2007). In the USA, edamame has potential as an easier to grow, better tasting, more nutritious substitute for lima beans (Konovsky et al., 1994; Zang and Boahen, 2007). It is a good source of protein and fiber, and also has high minerals and vitamins (Eupan, 2003). Moreover, it contains isoflavones, which are also known for many potential health benefits, including preventive effects on cancer, vascular disease, osteoporosis, menopausal symptoms, anti-diabetic effect, and cognitive function (Carson, 2010; Zang et al., 2011). It can also be used in salads, soups, stir fry, or stews (Khudson, 2003) or make filling of desert.

*Corresponding author. E-mail: btiroese@yahoo.com. Tel: +267 3650296. Fax: +267 3928753.

The soybean aphid, *Aphis glycines* Matsumura, has been established as a serious pest of soybean, *Glycine max* (L.) Merr., since it was first found in North America in 2000 and has caused millions of dollars in economic losses (Venette and Ragsdale, 2004). The insect is Nebraska's newest soybean pest and was first discovered in Nebraska soybean fields in mid-July 2002. It caused yield loss of over 20% in 2003 in Northeastern Nebraska (Hunt, 2004).

Soybean aphids are capable of reducing yield directly by feeding on young and green leaves, stems, and pods, which can result in a reduction in photosynthetic capacity (Myers et al., 2005), reduced pod number, seed size and quality, as well as total yield (Ostlie, 2001). The colonization of the soybean plants by the soybean aphid in the early vegetative growth stages has been reported to result in yield loss in excess of 50% (Wang et al., 1994). The aphids can also affect soybean yield indirectly by reducing seed protein content and by vectoring plant viruses such as alfalfa mosaic virus, soybean stunt, bean yellow mosaic, and soybean mosaic virus (Hill et al., 2001; Clark and Perry, 2002). The threat of soybean aphids to soybean production has triggered insecticide applications in a number of soybean fields in the US and Canada (Rutledge and O'Neil, 2006). Nearly 3 million ha of soybeans were sprayed to control the soybean aphid in the USA in 2003 (Landis et al., 2003). Until the recent discovery of plant resistance to the soybean aphid (Hill et al., 2004), chemical insecticide application was the only available means to control the pest. Planting date, climate and predators (Onstad, 2001) have also been found to affect soybean aphid populations.

Plant insect resistance and cultural practices are important components of an integrated pest management program to control insects (Mensah et al., 2005; Carson, 2010), as they are both cost effective and environmentally safe control methods (Hunt et al., 1995). Knowledge on the relative resistance of cultivars and on the impact of cultural practices is useful in the design of appropriate breeding procedures to develop resistant cultivars and for selection of appropriate varieties to plant in an area. However, information on the effects of cultivars and cultural practices on the lifecycle, behavior and on population dynamics of *A. glycines* in edamame soybean in North America have not been documented. This study was, therefore, initiated to gain understanding on the temporal and spatial population dynamics of *A. glycines* on two edamame soybeans in Nebraska. Results of this study will serve as baseline information in the management of the pest in edamame soybeans and selection of cultivars.

MATERIALS AND METHODS

Study site

A field experiment was conducted at the University of Nebraska-Lincoln's Agricultural Research and Development Center near Ithaca, Nebraska in 2004 and 2005. The field was surrounded by tall trees, shrubs and grasses on three sides and the other side was corn and soybean field.

Experimental design and planting

The experiment was arranged in a completely randomized design and was carried out twice in each of two years (2004 and 2005), with two planting dates each year. The treatment design was a 2 × 2 factorial, with cultivar and plant growth stage as the factors. Two edamame soybean cultivars, 'Envy' (64 days to harvest) and 'Butterbeans' (75 days to harvest), were planted, and the plants were observed at each of 8 possible developmental stages (V5 to R6 growth stages). The soybean vegetative (V5 and V6, 5 and 6th node with fully developed leaves, respectively) and reproductive growth stages (R1: beginning bloom, R2: full bloom, R3: beginning pod, R4: full pod, R5: beginning seed, and R6: full seed stage, respectively) were identified according to Fehr et al. (1971). In each planting date/year combination, each cultivar was replicated twice.

These cultivars were planted at two different planting dates to investigate the seasonal occurrence of *A. glycines*. A 35 × 47 m field area was divided into two parts corresponding to two planting dates for 2004 and 2005. The two cultivars were each planted in four row plots measuring 3 m by 9 m. A 1.5 m width strip of sweet corn (*Zea mays* L.) was planted at the margins of the field. This planting pattern was adopted throughout the experiment. The two planting dates were separated by 8 m wide strip, which was also planted in sweet corn to provide a buffer between plots.

Plots were seeded on 20 May and 3 June in 2004 and on 18 and 31 May in 2005. Planting was done at a rate of 371,000 seeds/ha. The distance between the seeds was 3.6 cm, and the rows were 75 cm apart. Any aphid infestation in the experimental plots occurred naturally. Plots were hand-weeded once every year when soybeans were at V5 growth stage to reduce the effects of weed competition.

Data collection

Aphid counts per plant were taken twice a week from the first time *A. glycines* was observed (V5 growth stage) to the end of the full seed stage (R6 growth stage). Developmental stages of the plants were also recorded throughout the growing period.

Data collection was done by direct observation of the number of *A. glycines* present on the plant. The aphid infestation levels were measured at the same plant growth stages for each cultivar. The number of *A. glycines* was determined by counting the aphids on the upper five trifoliate leaves of five randomly selected plants. There were two hundred and fifty plants per row on average. The first twenty and last twenty plants in a row were not counted to avoid plot margin effects.

Cumulative aphid-days

Cumulative aphid days (CAD) were calculated to estimate the total exposure of soybean plants to soybean aphid. The calculation was based on the number of aphids per plant counted on each sampling date. CAD was calculated with the following equation:

$$\sum_{n-1}^{\infty} = \left[\frac{x_{i-1} + x_i}{2}\right] \times t$$

where x is the mean number of aphids on sample day i, x_{i-1} is the mean number of aphids on the previous sample day, and t is the number of days between samples i - 1 and i.

Table 1. Mean number of *A. glycines* per plant in 2004 and 2005 at different planting dates.

Planting date	Season	Butterbeans		Envy	
		Mean	SE	Mean	SE
May 20	2004	79.81[a]	13.97	18.42[b]	14.73
June 3	2004	75.69[b]	13.24	74.63[b]	14.31
May 18	2005	76.73[a]	4.48	10.25[b]	5.17
May 31	2005	93.76[a]	11.76	18.53[b]	13.15

[a]Means for each date within a row followed by the same letter are not significantly different (P ≤ 0.05).

Table 2. Mean number of *A. glycines* per plant for 2004 and 2005 first planting date growth stages

Plant stage	2004 Season				2005 Season			
	Butterbeans		Envy		Butterbeans		Envy	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
R1	-	-	-	-	6.70[aB]	2.80	0.00[aB]	0.00
R3	11.50[aA]	7.56	0.00[aA]	0.00	14.91[aB]	7.72	6.45[aB]	3.75
R4	37.42[aA]	17.55	10.88[aB]	5.23	22.23[aA]	11.13	11.98[aB]	5.35
R5	71.85[bA]	21.24	15.17[aB]	8.25	71.72[bA]	18.50	15.49[aB]	9.23
R6	152.92[bA]	26.40	32.83[aB]	11.30	268.10[cA]	22.20	17.31[aB]	8.95

[a]Means having the same lower case letter within a column are not significantly different (P ≤ 0.05). [b]Means having the same upper case letter between columns for the same year are not significantly different (P ≤ 0.05).

Data analysis

The experimental data were analyzed as a split-plot design with cultivar as the main plot factor and developmental stage as the sub-plot factor. Population densities of *A. glycines* were analyzed using the PROC MIXED procedure (SAS Institute, 2004) to test for interactions between cultivar and developmental stage. When statistically significant interactions were present, simple effects of cultivar and plant stage were estimated and tested at the 5% level. In the absence of a statistically significant interaction, main effects of cultivar and plant stage were estimated and tested at the 5% level. Both main and simple effects were estimated using the LSMEANS option in PROC MIXED. The analysis was carried out separately for each planting date/year combination.

RESULTS

Effects of early planting on *A. glycines* infestation levels

The average number of soybean aphids per plant for the first planting date was significantly higher on the 'Butterbeans' cultivar than for 'Envy' in 2004 ($F_{1,33}$ = 7.96; P < 0.0013) and in 2005 ($F_{1,40}$ = 44.58; P = 0.0104) (Table 1). 'Butterbeans' had a higher level of aphid infestation than 'Envy' from the first day of sampling to the end of the R6 growth stage and significantly different level of infestations between the cultivars were recorded in R4, R5, and R6 growth stage (Table 2).

'Envy' reached the R6 stage on 2 August 2005 before a rapid population growth of soybean aphids started to occur. 'Butterbeans' reached the R6 stage on 11 August 2005 and it had a significantly greater aphid population as compared to 'Envy'. The average infestation of soybean aphid per plant was found to be significantly different among some of the 'Butterbeans' growth stages in 2005 ($F_{4,42}$ = 24.01; P = 0.0001) (Table 2). The R6 growth stage had a significantly higher average number of *A. glycines* per plant (268.10 ± 22.20) than the other growth stages, and the lowest infestations were observed at R1, which had a mean of 6.70 ± 2.80 *A. glycines* per plant. There were no significant differences in soybean aphid infestations observed for growth stages R1, R3, and R4. Similarly, some of the growth stages of 'Butterbeans' in 2004 for the first planting date were also observed to be significantly different ($F_{3,15}$ = 3.88; P = 0.03) in mean number of *A. glycines* per plant (Table 2). The density of the aphids reached a peak at the end of the R6 growth stage, with the density of the aphids reaching a peak by the end of the R6 growth stage.

In the first planting of the 2004 season, average aphid infestations per plant did not significantly differ for 'Envy' growth stages ($F_{3,13}$ = 7.03; P = 0.45). Similarly, in 2005, there were also no significant differences found in the numbers of aphids per plant at any 'Envy' growth stage ($F_{4,30}$ = 14.50; P = 0.5437) (Table 2). The soybean aphid population on 'Envy' remained low up to the R6 growth stage in 2004 and 2005. The aphid population was observed to increase after the end of the R6 growth stage

Table 3. Mean number of *A. glycines* per plant for 2004 and 2005 second planting date growth stages.

Plant stage	2004 Season				2005 Season			
	Butterbeans		Envy		Butterbeans		Envy	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
V5	23.75[aB]	11.35	15.50[aB]	6.63	10.51[aB]	5.73	2.08[aB]	1.53
V6	56.78[aA]	16.45	51.75[aA]	16.58	15.59[aA]	6.85	4.72[aA]	2.34
R1	36.20[aA]	12.40	26.45[aA]	11.65	22.16[aB]	8.90	7.73[aB]	2.95
R3	49.23[aB]	19.30	148.07[cA]	27.35	30.63[aB]	13.45	11.26[aB]	4.15
R4	168.52[bA]	27.38	97.90[bB]	24.56	194.99[bA]	27.53	15.31[aB]	5.24
R5	103.70[bA]	31.50	41.80[aB]	21.63	308.48[bA]	33.58	18.90[aB]	5.75
R6	31.20[aA]	18.20	21.45[aA]	9.51	23.34[aB]	9.47	52.40[aA]	14.56

[a]Means having the same lower case letter within a column are not significantly different (P ≤ 0.05). [b]Means having the same upper case letter between columns for the same year are not significantly different (P ≤ 0.05).

(not recorded). 'Envy' reached the edamame harvest stage (R6) before the rapid increase of the soybean aphid population in both years.

Effects of late planting on *A. glycines* infestation levels

During the 2004 season, the average soybean aphid count per plant for 'Butterbeans' and 'Envy' did not significantly differ ($F_{1,32}$ = 1.34; P = 0.56) (Table 1). However, during the 2005 season, the second planting date period showed significant differences in *A. glycines* incidence between the two cultivars ($F_{1,53}$ = 34.66; P = 0.0001). 'Butterbeans' had a significantly higher incidence of *A. glycines* per plant than 'Envy.' 'Envy' still had green leaves, but 'Butterbeans' was observed to have greener and younger leaves as compared to 'Envy'.

Some of the growth stages of 'Butterbeans' in 2004 were found to be significantly different in average soybean aphid infestation levels per plant during the second planting period ($F_{5,32}$ = 38.07; P = 0.025) (Table 3). Some of the 'Butterbeans' growth stages in 2005 were also observed to significantly differ ($F_{6,52}$ = 31.80; P = 0.0001) in average soybean aphid populations per plant (Table 3). Higher soybean aphid counts per plant were observed at R4 growth stage in 2004 and at R5 growth stage in 2005. In the 2005 season, the aphid populations were at low levels on 'Butterbeans' until the end of the R3 growth stage (Table 3). Populations started to increase from 11 August (Figure 2) at R4 stage and reached a peak at the R5 growth stage. The soybean aphid populations started to decline by the end of the R5 growth stage, and the numbers were low by the beginning of the R6 growth stage.

Some of the 'Envy' growth stages in 2004 were significantly different in soybean aphid infestation per plant during the second planting date period ($F_{5,32}$ = 28.84; P = 0.030) in aphid counts per plant. The R3 stage had a higher mean number of *A. glycines* per plant (148.07 ± 27.35), and the lowest mean number of *A. glycines* per plant was observed at the V5 growth stage (15.50 ± 6.63) in 2004. However, in 2005 'Envy' growth stages were not significantly different ($F_{6,52}$ = 22.39; P = 0.4239) in soybean aphid infestation per plant (Table 3).

In 2005, the *A. glycines* populations were relatively low on 'Envy,' until the end of the R5 growth stage during the second planting period. *A. glycines* populations started to increase at the R6 growth stage (Figure 2), and reached their peak population after the R6 growth stage. 'Envy' reached the R6 harvesting stage prior to the upsurge in the *A. glycines* population.

There was a significant difference in average population of soybean aphids per plant in 2005 between the two cultivars ($F_{6,40}$ = 47.01; P = 0.0001) for some of the growth stages observed (Table 3). 'Butterbeans' had higher *A. glycines* incidence than 'Envy' at R4 and R5 growth stages. It should be noted that 'Butterbeans' reached their R6 stage after the upsurge of *A. glycines* population later in the season, and 'Envy' reached its R6 stage before the upsurge. A similar observation of aphid occurrence was noted in the 2004 season.

Cumulative aphid-days

The mean cumulative aphid-days for 'Butterbeans' and 'Envy' for both first and second planting periods in 2005 were not significantly different (Figure 2) from 14 July ($F_{3,33}$ = 0.01; P = 0.99) up to 9 August ($F_{3,33}$ = 0.54; P = 0.66). However, starting on 11 August, the mean cumulative aphid-days were observed to differ significantly ($F_{3,33}$ = 14.84; P < 0.0001), with the highest incidence recorded on 'Butterbeans' for the second planting period, and the lowest incidence observed on 'Envy' for the first planting period.

DISCUSSION

A. glycines overwinter as eggs on their primary host, buckthorn (*Rhamnus* species) (Yoo et al., 2005). In the summer, the aphids move in search of secondary hosts (cultivated soybeans), where many generations of asexual reproduction occurs (Ragsdale et al., 2004). These various summer movements are thought to be the likely source of infestations in Nebraska (Hunt, 2004). Colonization and build-up of aphid population are affected by different factors including planting date, predators, host plant resistance, insecticides temperature, rainfall, wind velocity and direction, and the amount of vegetation (Onstad, 2001; Underwood, 2004). Significant yield loss (8 to 25%) occurs when the soybean plants are heavily infested by the aphid during the early reproductive stage (DiFonzo and Hines, 2002). This study revealed the influence of plant phenology at the time of infestation. Aphid infestation in early planted started after the early maturing 'Envy' cultivar reached R4 stage in both 2004 and 2005. Early planted 'Butterbeans' cultivar also escaped aphid infestation at vegetative stage. Lin et al. (1992) showed that the soybean aphid colonizes soybeans in China at the early vegetative stage. It is, therefore, not surprising that significantly lower aphid infestation was recorded in the early maturing envy cultivar and early planted 'Butterbeans' in both years as physiologically mature cultivars have less nutritious vegetative material available to attract and support an aphid population than younger ones (Dixon, 1970). Previous studies also reported that the intrinsic rate of increase of soybean aphid was found to decline as soybean plants aged (Van den Berg et al., 1997; Ragsdale et al., 2007). Hence, early planting of edamame is recommended, because the crop utilize the entire growing season and achieve physiological maturity before a serious aphid infestation. Earlier studies have reported that earlier planting dates in soybeans have produced larger yields (Cox et al., 2008; Robinson et al., 2009; Carson, 2010). Our result also shows that Envy and early planting is the best combination. However, awareness of the yield potential and associated benefits of this vegetable soybean should be taken into consideration as envy was reported to be a low yielding cultivar (Zhang and Boahen, 2007).

The relatively greater growth and fecundity rates of aphids in 'Butterbeans' are likely to be attributed to young and green leaves in this variety. 'Butterbeans' was observed to have vegetative growth (leaves) overlapping with reproductive growth (flower, pods, and seeds), which is a characteristic of indeterminate varieties. There is no information on the two cultivars that qualifies them as either determinate or indeterminate. As younger leaves continue to be produced, the plants become more attractive to insects, because the leaves are still young and green. However, we observed that 'Envy' did not continuously produce young leaves through the reproductive stages, which is a characteristic of determinate varieties. Several studies have postulated that plant growth form may affect the seasonal changes in plant nutritional quality (Schultz et al., 1982; Meyer and Montgomery, 1987; Stamp and Bowers, 1990; Bowers et al., 1992; Jordano and Gomari, 1994). The nutritional quality of 'Envy,' with its determinate growth habit, has been observed to decrease more rapidly and more severely with plant phenological age than for the indeterminate 'Butterbeans'. This can be caused by the difference in age structure of the leaves (Schultz et al., 1982; Bowers et al., 1992). As foliar nutritional quality decreases with leaf age for cultivars such as 'Envy,' due to maturation and senescing processes, free-growth species such as 'Butterbeans' are believed to maintain foliar nutritional quality at higher levels, because of the continuous flush of young, nutritious leaves, contrary to the determinate species where all leaves age simultaneously from the start of the season with no rejuvenation. There is no information available on economic threshold levels of soybean aphid on edamame soybeans as it is with grain soybeans. But, because edamame is marketed as green vegetable, the pest tolerance level is low and this can also similarly imply low economic threshold levels of soybean aphids on edamame. The average economic threshold level for soybean aphid on grain soybean is 273 aphids per plant for R6 and later stages (Ragsdale et al., 2007). More research is needed to measure the economic threshold on edamame up to R6 stage (harvesting stage).

CAD summed the aphid days which were accumulated during the growing season and this provides a measure of the seasonal aphid exposure that a soybean plant experiences. CAD provides a measure of aphid abundance overtime on soybean plants. Soybean aphids on 'Butterbeans,' in both first and second planting period, increased from 11 August to end of August, and thereafter started to decline. The aphids were last observed on 'Butterbeans' for first planting on 23 August, but for second planting period, *A. glycines* continued to be observed on 'Butterbeans' up to 1 September. Similar cumulative aphid day patterns were observed in 2004 (Figure 1). The mean cumulative aphid-days for 'Envy,' for both first and second planting periods, remained low from 14 July up to 9 August. Beginning on August 11, the cumulative aphid days for 'Envy' during the second planting period increased slightly to 16 August, and thereafter no aphids were observed. During the first planting period, 'Envy' had no aphids from 11 August up to the end of Mid-August. The 2004 season had a similar pattern of cumulative aphid days as the 2005 season. Therefore, 'Butterbeans' were exposed to soybean aphids for a longer time as compared to 'Envy', hence, are able to be affected more by the aphids as compared to the 'Envy'.

Conclusion

Conclusively, the study revealed that planting date and

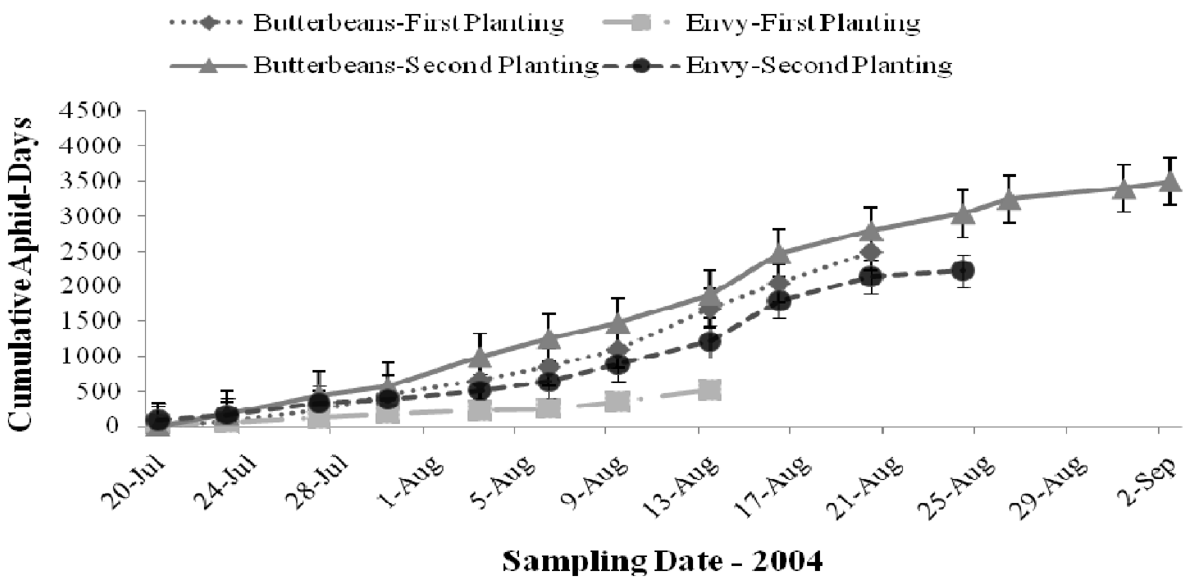

Figure 1. Mean cumulative aphid-days for 'Butterbeans' and 'Envy' for both first and second planting periods in 2004.

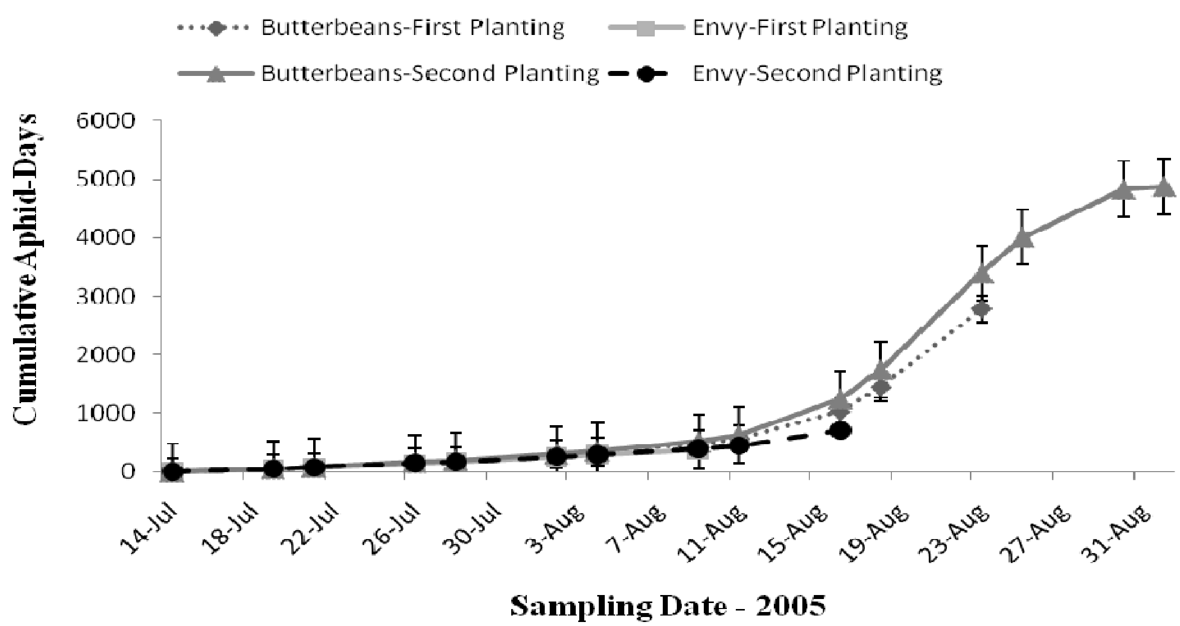

Figure 2. Mean cumulative aphid-days for 'Butterbeans' and 'Envy' for both first and second planting periods in 2005.

cultivar choice, based on maturity period, can affect the soybean aphid population growth on edamame vegetable soybeans. It can be recommended that planting early and using early maturing cultivars when the area has an aphid population build-up likelihood later in the season. 'Butterbeans' showed significantly higher aphid population growth than 'Envy' for both first and second planting periods in 2004 and 2005. The difference mainly showed at late reproductive growth stages, R5 and R6, where 'Butterbeans' had more aphid population growth than 'Envy' for both planting periods in 2004. Therefore, it can be hypothesized that the difference in the aphid population levels between the two cultivars may be the result of their physiological differences.

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