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Evaluation of Apple and Orange Fruits as Food Sources for the Development of *Halyomorpha halys* (Hemiptera: Pentatomidae)

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썩덩나무노린재의 발육을 위한 먹이원으로 사과와 밀감의 평가

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ABSTRACT: *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is a typical polyphagous stink bug causing losses in several host plants including leguminous crops and fruits. Nutritional status of fruits such as apple and orange for the development of *H. halys* is not yet clear. We evaluated fruits of apple and orange with or without soybean-peanuts as food sources to investigate development, mortality and fecundity of the stink bug in a controlled condition. Those only fed on water could not develop into third instars. *H. halys* could not develop into fourth instar on apple only food. However, on an orange only food, 14% of *H. halys* emerged as adults. Those fed on orange only food had the longest development period (74.2 d). Total mortality of those fed on foods consisting of soybean-peanut ranged from 38 to 44%. In an average a female laid 169~190 eggs in a lifetime and those eggs had 81~83% hatchability. Results indicated that the apple fruit as a solo food source is an incomplete food for *H. halys* development while orange could support development of only few of the bugs.

Key words: Food, Development, Mortality, Stink bug, Fecundity

초 록: 썩덩나무노린재는 두과작물에서 과실에 이르기까지 다양한 작물을 가해하는 대표적인 다식성 노린재의 일종이다. 과실이 썩덩나무노린재의 발육에 필요한 영양원인지 불필요한 영양원인지 아직도 분명하지 않다. 그리하여 사과와 오렌지의 단독먹이 및 콩-땅콩과의 먹이조합에 따른 썩덩나무노린재의 발육, 사망률 및 증식률을 평가하였다. 썩덩나무노린재 약충은 사과단독먹이에서만 높은 사망률로 성충으로 발육하지 못하였다. 물만 제공한 것은 3령으로 발육하지 못하였고, 사과단독먹이에서 4령으로 발육하지 못하였다. 하지만 오렌지단독먹이에서 14%가 성충으로 우화하였으나, 약충의 발육기간이 약 74일로 가장 길었다. 콩-땅콩과 조합한 먹이에서 노린재의 사망률은 38~44%를 나타내었다. 총 산란수는 169~190개 였으며, 부화률은 81~83%이었다. 따라서 사과는 썩덩나무노린재의 발육에 불완전한 먹이 이었으나, 오렌지는 발육이 지연되기는 하였으나 이루지는 것으로 평가되었다.

검색어: 먹이, 발육, 사망률, 노린재, 증식률

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is native to Korea, Japan, and China. As an invasive pest, the stink bug was identified for the first time in 2001 in Pennsylvania (Bernon, 2004) long after its introduction, and it has spread throughout the Mid-Atlantic States, Oregon, and California (Nielsen and Hamilton, 2009). Also, presence of *Halyomorpha sp*. in Europe was reported by Aukema and Rieger (2006) and isolated populations found to exist in Switzerland and Canada (Leskey et al., 2012).

H. halys is one of the major polyphagous pentatomids with a wide host range ranging from ornamental shrubs to cultivated crops (Hoffmann, 1931; Hoebeke and Carter, 2003; Bernon, 2004). It feeds on coniferous cones and is considered a pest in cedar and cypress seed farms in Japan (Funayama, 2005; Kiritani, 2007). More importantly, *H. halys* is known to occur in fruit

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trees in Japan and Korea (Chung et al., 1995; Choi et al., 2000; Funayama, 2002), and is the dominant pentatomid pest in yuzu [*Citrus junos* Siebold (Sapindales: Rutaceae)] as recorded at Koheung in Korea (Choi et al., 2000). Among citrus fruits, sweet orange (Yu and Zhang, 2007) and satsuma mandarin (Oda et al., 1980) have been also reported as host crops of *H. halys*. A host preference study revealed that adult *H. halys* preferred elaeagnus, ligustrum, and orange to other tested hosts (Poplin, 2013). However, fruits such as apples are considered as an early season host for *H. halys* emerging from overwintering sites (Funayama, 2002). Apple trees may not be a primary host since apple fed females produced fewer eggs; however, the apple fruits are important when other, more suitable fruits or hosts are scarce (Funayama, 2004).

Since apple and citrus fruits have greater economic importance and occurrence of *H. halys* and its damage in apple and citrus fruits have been reported by several studies, we carried out this study to determine the suitability of fruits only food for the developmental success of *H. halys*. The findings may help to rectify the host status of the orange and apple fruits for *H. halys*, and subsequently would help develop appropriate strategy to manage the occurrence of *H. halys* on the apple and orange fruit orchards.

Materials and Methods

Insect rearing

H. halys adults and nymphs were collected from sesame fields of National Institute of Crop Science, Miryang, Korea in 2012. Both nymphs and adults collected from the fields were brought in laboratory and placed inside an acrylic rearing cage (40 × 40 × 40 cm) ventilated with fine mesh on the lateral sides. Soybean plants grown in glasshouse and soybean (*Glycine max* [L.] Merr.) plus raw peanut (*Arachis hypogaea* L.) seeds were provided as food sources. Insects were reared on a 16 hour photoperiod (16:8 h L: D) at 25±2°C, and 50-55% RH.

Developmental rate, mortality and fecundity of *H. halys*

The effect of different diets on developmental time was examined at 25±1°C, photoperiod 16:8 h (L: D) and 50 to 55% relative humidity (RH). Eggs collected from the laboratory colony were placed inside a breeding dish (14 Dia × 2.5 H cm). After the eggs hatched and the first instar nymphs, which do not feed, molted to second instar they were randomly chosen and placed individually into rearing containers with six different diets and were compared for development time. The diets were (1) water (tap water on a soaked sponge placed in a cup) (2) water plus dried soybean-peanut seeds (3) water plus orange (*Citrus sinensis* [L.]) (4) water plus dried soybean-peanut seeds plus orange (5) water plus apple (*Malus sylvestris* var. domestica [Borkh.] Mansf.) and (6) water plus dried soybean-peanut seeds plus apple. Soybean and peanuts used in the study were dried to a consistent moisture level (< 14%). Both orange and apple fruits used in the experiment were ripe.

Individuals were checked every 24h for development or mortality. Water was replaced every other day and diets were changed thrice weekly. At least 34 individuals for each diet were individually tested. Development was confirmed by the presence of exuvia and nymphal characteristics as outlined in Hoebeke and Carter (2003). Stage-specific mortality of each nymphal stage was calculated as [Sx/lx], where Sx = the number of individuals dying at stage x and lx = the number of individual alive at the beginning of stage x. Total mortality was calculated as [Sx/lo], where lo = the starting number of individuals (Wittmeyer and Coudron, 2001). At the final molt to adult, all individuals were sexed.

Providing the same diet, each male *H. halys* was paired with a female and placed in the incubator to observe fecundity. At least eight mated adult females for each diet combination were tested for the fecundity. Since *H. halys* were found to lay eggs successfully on underside of the lid of the container, no specific oviposition substrate was provided.

Statistical analyses

Data for developmental time and fecundity were square root transformed before analysis. Also, data for percent hatched eggs was arcsine transformed before analysis. Developmental time, fecundity and percent hatched eggs were analyzed by PROC GLM (SAS, 2002), with Tukey's test for mean separation. Mortality was compared among diets using a Chi-square test of a contingency table and a post-hoc multiple comparison test analogous to the Tukey's test (Zar, 2010).

Results

Developmental time, mortality, fecundity and hatchability

In second instar (F = 338.66, df = 4, 115, P < 0.0001), third instar (F = 376.24, df = 3, 84, P < 0.0001), fourth instar (F = 323.62, df = 3, 70, P < 0.0001), and fifth instar (F = 652.53, df = 3, 67, P < 0.0001), diets supplemented with soybean-peanut seeds showed shortest development time compared to those fed on fruits only diet. Among the different nymphal developmental stages, the longest development time of *H. halys* was observed during the period of 5th instar. On orange only diet, *H. halys* took 74.15 d to develop into adult (Table 1).

The effect of diet on the survival of *H. halys* was significant in second star (χ^2 = 86.46, df = 5, P < 0.001); nymphs on water only diet could not molt into third instar and those fed on fruits only diet had higher mortality. *H. halys* could not develop into fourth instar in apple plus water diet and showed 100% mortality in third instar, and there was 86% mortality of those fed on orange plus water (χ^2 = 36.81, df = 5, P < 0.001). Alike other instars, in fourth instar too, those fed fruit only diet showed higher mortality (χ^2 = 19.52, df = 5, P < 0.001), but those molted into the fifth instar could successfully develop into adult (χ^2 = 1.95, df = 5, P = 0.584). Mortality of those fed on diets consisting of soybean-peanut ranged from 38 to 44%. On orange only diet, a 14% of *H. halys* could emerge as adults (Table 2).

Fecundity was compared among those fed on the diets consisting soybean-peanut with and without fruits, because those fed on fruits only diet failed to reproduce. No significant difference was found among the diets (F = 0.49, df = 2, 23, P = 0.620). In an average each female was found to lay 168 to 190 eggs in a lifetime. Similarly, no difference was found in the percent eggs hatched (F = 0.87, df = 2, 23, P = 0.434). Hatchability was found to range from 81 to 83% (Table 3).

Table 1. Mean developmental time (days±SE) of *H. halys* from second instar to adult stage fed on different food sources (incubated at 25℃)

Food sources	Sample size (n)	Instars				Total
		2nd	3rd	4th	5th	(2nd instar to adult stage)
Water	36	-*	-	-	-	-
Water+Soybean-peanut	36	9.97±0.19b	7.23±0.14b	7.50±0.11b	10.86±0.13b	35.56±0.35b
Water+Orange	35	16.65±0.12a	15.00±0.23a	16.63±0.18a	25.88±0.35a	74.15±0.48a
Water+Orange+Soybean-peanut	37	9.67±0.12b	7.26±0.09b	7.88±0.11b	10.61±0.11b	35.41±0.24b
Water+Apple	35	16.60±0.16a	-	-	-	-
Water+Apple+ Soybean-peanut	34	9.69±0.04b	7.41±0.04b	8.00±0.04b	10.58±0.05b	36.68±0.07b

Means followed by same letters in the same column are not significantly different (P<0.05).
*Data not recorded because of 100% mortality.

Table 2. Stage specific and total mortality (proportion) of *H. halys* developmental stages fed on different food sources (incubated at 25℃)

Food sources	Stage specific mortality				Total mortality
	2nd	3rd	4th	5th	
Water	1.00	-	-	-	1.00
Water+Soybean-peanut	0.14	0.16	0.15	0.05	0.42
Water+Orange	0.34	0.43	0.62	0.00	0.86
Water+Orange+Soybean-peanut	0.19	0.10	0.07	0.08	0.38
Water+Apple	0.71	1.00	-	-	1.00
Water+Apple+Soybean-peanut	0.24	0.19	0.10	0.00	0.44

Table 3. Lifetime fecundity (Mean±SE) and hatch rate (Mean±SE) of *H. halys* fed on different food sources (incubated at 25°C)

Food sources	Mated female (n)	Fecundity	Hatch rate
Water+Soybean-peanut	8	171.13±13.43a	0.83±0.01a
Water+Orange+Soybean-peanut	9	189.80±16.38a	0.82±0.01a
Water+Apple+Soybean-peanut	9	168.50±13.35a	0.81±0.01a

Discussion

This is probably the first laboratory study that reports inability of *H. halys* nymphs to develop into adult on apple fruit. Though few of them developed to adult on orange only diet, they took almost twice longer period to become an adult ㄴto 44% on normal leguminous diet. In agreement to our study, Nielsen et al. (2008) also reported mortality of *H. halys* up to 39% when reared at 25°C. Although slower, but development on orange only diet in a controlled laboratory condition raise a concern for the citrus growers. Because, presence of other plant parts and alternative nutritional resources in the field conditions may support their optimal development. However, failure to develop on apple fruits suggests that occurrence of the bugs on apple may be temporary and they require other host sources to complete their life cycle and to reproduce successfully. Discrepancy in the *H. halys* performance on these two fruits may be because of different nutritional contents. Vitamin C content in oranges has been reported to be significantly higher than that in apple fruits (Lee and Kader, 2000). Nevertheless, fruits at different critical growth stages may have different nutritional source which this study has not addressed.

Cultivated fruit species have been reported to be not suitable for full development of a pentatomid *Plautia crossota stali* Scott (Oda et al., 1980; Shiga and Moriya, 1984). Also, an alydid, *Riptortus pedestris* (F.) failed to develop on fruits only diet (Kim and Lim, 2012). Funayama (2002), however, found that adult *H. halys* collected from overwintering sites had mature eggs in the ovaries and they laid eggs when released to apple orchard. Apple trees supported the development of offspring. Funayama (2004) reported survival and sexual maturation of adult *H. halys* on apple trees with fruits, but opined that the fruit trees may not be the optimal hosts for nymphal development. Because of the occurrence of very few number of *H. halys* nymphs in the apple orchard, Nielsen and Hamilton (2009) also suggested that apple trees may not be suitable for its nymphal development. Lee et al. (2013) suggested that reproductive development of *H. halys* is affected by photoperiod, temperature, and diet. Most of the fruits for *H. halys* were categorized as food plants (Oda et al., 1980 as cited in Lee et al., 2013); food plants were those on which only adult population were observed but not the eggs and nymphs. This study has clarified that the fruits of apple do not support nymphal development of *H. halys,* but orange fruits do support development of few bugs though adults developed from orange only diet were not capable of laying eggs. Funayama (2006) found successful growth and development of *H. halys* on carrot, but mentioned that they took significantly longer duration to develop into adult compared to those supplemented with soybean-peanut diet similar to the development time of *H. halys* fed on orange in our study. The role of nutritional content of the hosts for successful development and reproduction could be another important research work to be conducted to understand the discrepancies in developmental success of *H. halys* among similar and distantly related host species.

Fecundity and hatchability was not affected whether or not fruit was provided with soybean-peanut diet. Vitamin C is known to improve fecundity and egg eclosion in a pentatomid (Noda and Kamano, 2002). Fruits contain vitamin C but other constituents such as tannins may also play role as existence of high concentration soluble tannins in sweet persimmon was found to inhibit reproduction in *R. pedestris* (Park et al., 2004).

Results from this study suggest that this stink bug cannot complete its development on apple fruit. Though orange fruit supported the development up to the adult stage, it is not the optimal diet source of *H. halys* as the developed adults could not reproduce. Thus, apple and orange fruits are incomplete sources of nutrition for *H. halys*; adults may use the fruits after emerging from diapause and then as they prepare to enter diapauses in the late season (Funayama, 2004; Nielsen and Hamilton, 2009).

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