

Morphological and Behavioral Comparison between Males and Females of Arrowhead Scale, *Unaspis yanonensis* (Homoptera: Diaspididae) on Citrus Leaves

Yon Dong Seo¹, Sang Cheol Kim¹, Saem Lee Park¹, Kyung San choi²
and Dong Soon Kim^{1*}

¹제주대학교 식물자원환경전공

²난지농업연구소

화살깍지벌레 암컷과 수컷 형태적 특징 및 정착행동 비교

서연동¹, 김상철¹, 박샘이¹, 최경산², 김동순^{1*}

¹Majors in Plant Resource Sciences & Environment, Cheju National University

²National Institute of Subtropical Agriculture, RDA

ABSTRACT

Arrowhead Scale, *Unaspis yanonensis* Kuwana (Homoptera: Diaspididae) is a destructive pest on citrus trees in Jeju. Because the crawlers (hatched nymphs) are the only spreading stage, the knowledge for their settling behavior and morphological changes is very important to facilitate control measures and an experimental design. The body color of hatched nymphs (crawlers) of *U. yanonensis* was different between the females and males. The majority of female crawlers showed a deep yellow color during the period from their hatching to the early settlement state, while pale yellow for male crawlers during the same life stage. The female nymphs produced a two long waxy filament on their head end within a few hours

after settlement, and then went through two nymphal stage without a protective scale cover. Whereas, settled male nymphs produced a short curled dense waxy filament on the dorsum, and then secreted a three white cotton-like wax strips on the dorsal surface. The protective scale cover of female nymph, which is a permanent female's shield, was gradually formed from the end of the exuviae of the 2nd nymph. The development stage of *U. yanonensis* female was divided into two stages of non-protective cover stage and protective cover stage according to the development of waxy scale cover. Also, the settling behavior between males and females was very different. Male crawlers settled down in clustered groups on leaves with nearly regular spacing each other, and not touching one another. Female

* Corresponding author : Dong Soon Kim, 제주시 제주대학로 66 제주대학교
Tel. 064-754-3312, E-mail dongsoonkim@cheju.ac.kr

crawlers did not form a cluster, and settled down in solitary, touching each other or sometimes overlapping together. In a two-leaf choice test by hatched crawlers, females moved freely and settled down on the other side leaf as well as the leaf where they were born. However, males took their position at the leaf where their mothers had lived. Such a dispersal behavior was observed in the field. Female crawlers dispersed from overwintered leaves to spring leaves, and from spring leaves to summer leaves sequentially.

Key words : *Unaspis yanonsis*, Citrus, Settling behavior, Dispersal, Body color

INTRODUCTION

Arrowhead scale, *Unaspis yanonensis* (Kuwana) (Homoptera: Diaspididae), is an important citrus pest in Asian regions, including Korea, Japan, and China (Ohkubo, 1980; Blackburn and Miller, 1984). *U. yanonensis* primarily feeds on the foliage, small diameter stems, and fruit of citrus hosts. Also, severe attacks during the spring (threshold of 8 females per leaf) can result in tree death within a year (reviewed in Ohkubo, 1980).

U. yanonensis overwinters primarily as fertilized adult females in Jeju, Korea, although a small portion of the population may overwinter as second female instars, or as immature males in warmer regions of other countries (Kuwana 1923; Murakami, 1970). The adult *U. yanonensis* females lay eggs beneath their scale covers (Okudai *et al.*, 1966). The eggs hatch within a few hours or as soon as they are laid (Korenaga, 1983), because they are deposited in the condition of almost completed embryonic development (Takezawa and Aihara, 1962). Newly emerged, the 1st

nymphs (crawlers) disperse and settle down within a few hours after hatching (Ohkubo, 1978; Korenaga, 1983). The crawler stage (hatched nymphs) is the only one in which a scale infestation can spread. All other developmental stages, except adult males, are immobile and attached to a single feeding site under a protective scale covering. Females go through two nymphal stages and reproduce in the third nymphal stage, consequently, the adult stage, following mating. The males have five developmental stages: the first and second nymphal instars, prepupa, pupa, and alate imago (Kuwana, 1923).

Unaspis yanonensis females have a protective scale cover, which may prevent the effects of insecticides based on contact toxicity. Petroleum oil is a representative material that kills scales via a suffocative mode, and it is frequently used in Jeju citrus orchards managed by environment-friendly practices. Hence, it is of primary importance to determine the occurrence period where the control effect of *U. yanonensis* can be maximized; consequently, the stages prior to attaining coverage with the thick protective scale. To predict the specific stage for control in the field, it is necessary to know the morphological change of *U. yanonensis*. Also, understanding dispersal behavior is helpful for the setting of control measures as well as the fundamental study of dispersal ecology. Thus, this study was conducted to examine the settling behavior and morphological changes of *U. yanonensis* on citrus.

MATERIALS and METHODS

Observation of morphological characteristics and settling behavior

A pieces of citrus leaf (30×30 mm) were placed upside down on cotton wool saturated with water in a petridish (dia. 90mm). The pieces of citrus leaf included single female of *U. yanonensis*. Observation of >20 females were made. Daily observations were made to examine the morphological characteristics and settling behavior of *U. yanonensis* under a binocular-microscope with 10 to 100x.

Dispersal of hatched nymphs

Two-leaf choice test. Citrus shoots infested with overwintered *U. yanonensis* adult females were collected on late April, 2006 in an abandoned orchard. The shoots were cut in a 15 cm long and then all leaves were removed except two leaves (Fig. 1). Five females were allowed on one side leaf, while all females were removed on the other one side leaf. The shoots attaching two leaves were inserted into a resinoid block saturated with tap water in a plastic container, and held at 24°C in the laboratory. The settled crawlers on both leaves were examined after two weeks from the 1st emergence of crawlers.

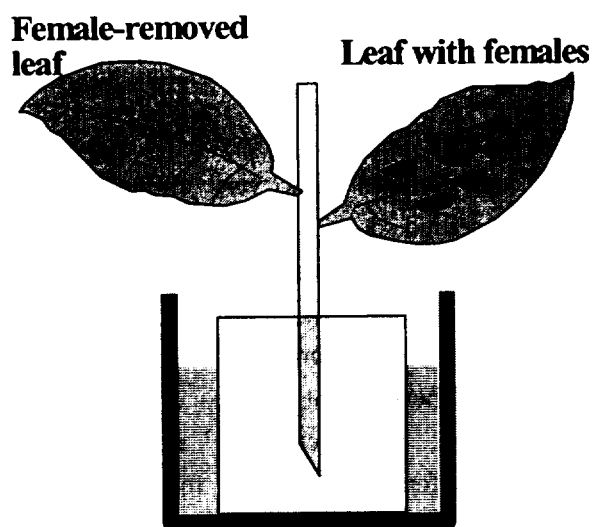


Fig. 1. A device for two-leaf choice test of hatched *U. yanonensis* crawlers.

Seasonal dispersal of female and male crawlers. Citrus leaves were classified into three categories according to their emergence season (generation of leaves) as seen in Fig. 2. The overwintering generation of *U. yanonensis* females is only found on the overwintered leaves which have emerged and grown in the previous year, because spring and summer leaves appear in the current year. The dispersal of the 1st generation crawlers, which were produced from overwintered *U. yanonensis* adult females, was monitored on the overwintered leaves and new spring leaves in the spring season. The 2nd generation crawlers were counted on the spring leaves and summer leaves in late summer to autumn season. Fifteen to 30 leaves in each leaf category were sampled in an abandoned orchard according to sampling dates. The collected leaves were put in a paper bag and then sealed in a vinyl bag (20×30 cm), and placed in a refrigerator at 4°C until examined. The numbers of *U. yanonensis* on the collected leaves were examined under a binocular-microscope with 15 to 30 x magnification.

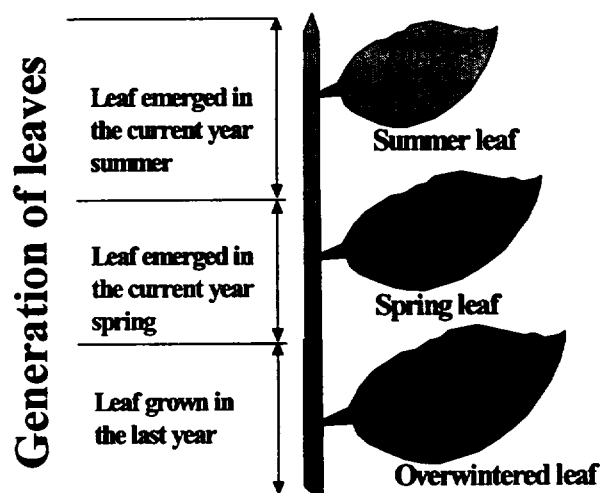


Fig. 2. Classified category of citrus leaf generation according to their emergence season.

RESULTS

Color and morphological change

The body color of hatched nymphs (crawlers) of *U. yanonensis* was different between the females and males. The majority of female crawlers showed a deep yellow color during the period from their hatching to the early settlement state (Fig. 3: ① - ③), while pale yellow for male crawlers during the same life stage (Fig. 4: ①). The female nymphs produced a two long waxy filament on their head end within a few hours after settlement (Fig. 3: ②), and then secreted an arch-like loose wax frame over their body in a horizontal way (Fig. 3: ③). Whereas, settled male nymphs produced a short curled dense waxy filament on the dorsum (Fig. 4: ②), and then secreted a white cotton-like wax strips in three directions from the body end (Fig. 4: ③), consequently resulting in elongated scale cover with three longitudinal wax strips on the dorsal surface (Fig. 4: ④). The integument of the female 1st nymphs became hardened before the 1st molting (Fig. 3: ④ & ⑤). The body of the 2nd nymph female began to be pushed out from under the exuviae of 1st nymph with forming simultaneously a dome-like wax membrane over the growing body (Fig. 3: ⑥ - ⑨). The wax membrane disappeared with the hardening of the integument of 2nd nymphs. The protective scale cover of female nymph, which is a permanent female's shield, was gradually formed from the end of the exuviae of the 2nd nymph (Fig. 3: ⑩ - ⑬). A completed scale cover of female adult is provided in Fig. 3 ⑭ - c. Here, the exuviae of 1st and 2nd nymph are seen (indicated by a & b), respectively. Adult males were small, two-winged insects that emerged from the elongated scale covers after the last molts (Fig. 4: ⑤).

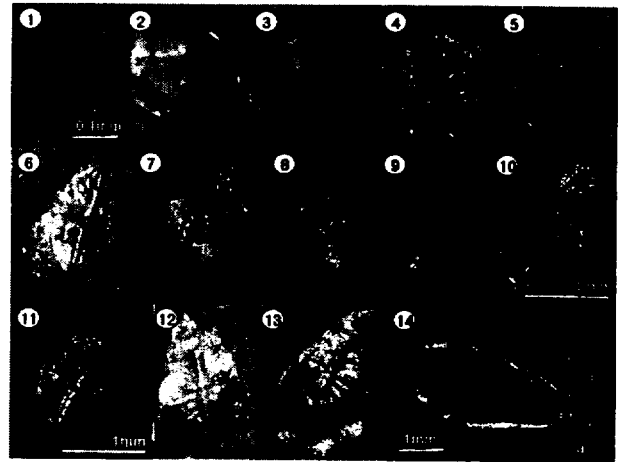


Fig. 3. Morphological changes of *U. yanonensis* females. The exuviae of 1st and 2nd nymph were indicated by a and b, respectively. The c is the permanent scale cover of female.

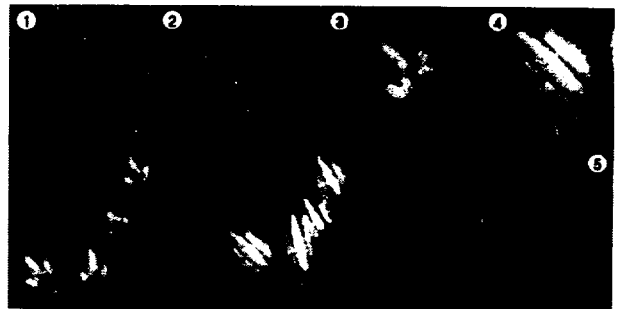


Fig. 4. Morphological changes of *U. yanonensis* males. The exuviae of 1st nymph was indicated by a.

Settling behavior

The settling behavior between *U. yanonensis* males and females was very different. Male crawlers settled down in clustered groups on leaves with nearly regular spacing each other, and not touching one another. Also, males seldom moved out from the leaf where they were born. There might be tens or as many as hundreds or more crawlers in a cluster, according to female density in the leaf. Female crawlers did not form a cluster, and settled down in solitary, touching each other or sometimes overlapping together. Also, females

Table 1. Number of *U. yanonensis* nymphs per leaf (Mean \pm SE) settled down on different leaf age in the field.

Sample date	Females on each leaf's category			Males on each leaf's category		
	Overwintered leaf	Spring leaf	Summer leaf	Overwintered leaf	Spring leaf	Summer leaf
9 Jul. 2006	128.1 \pm 23.61	30.8 \pm 5.61	-	620.3 \pm 135.98	0.0	-
14 Jul. 2006	177.8 \pm 27.28	50.5 \pm 7.34	-	555.0 \pm 102.86	0.0	-
9 Sep. 2006	-	247.2 \pm 47.02	21.3 \pm 4.72	-	945.2 \pm 176.92	0.0
14 Sep. 2006	-	215.3 \pm 32.83	18.9 \pm 3.04	-	894.4 \pm 149.57	0.0

¹The leaf samples were not taken.

prefer to locate on around the main vein of leaf, the marginal part of leaf, and leafstalk, but male did not.

Dispersal of hatched nymphs

Two-leaf choice test. In a two-leaf choice test by hatched crawlers, females freely moved and settled down on the other side leaf, although much portion of hatched females were observed on the leaf where they were born (Fig. 5). However, all males were only found on the leaf where *U. yanonensis* adult females presented.

Seasonal dispersal of female and male crawlers. *U. yanonensis* crawlers showed different dispersal behavior between males and females in terms of the selection of leaves for

their settlement (Table 1). On July samples, considerable numbers of female crawlers were found on spring-emerged new leaves: $\approx 80\%$ on overwintered leaves and $\approx 20\%$ on spring leaves. However, no males were found on the spring leaves, and all the males were on overwintered leaves on which overwintered adult females were found. For the 2nd generation crawlers which were born on spring leaves or probably on overwintered leaves (see September samples), the same dispersal patterns with those on July samples were observed. A part of the 2nd generation female crawlers ($\approx 8\%$) left the leaves on which they were born, and moved to summer-emerged new leaves, while male crawlers were discovered from only the spring leaves on which the 1st generation adult females were found.

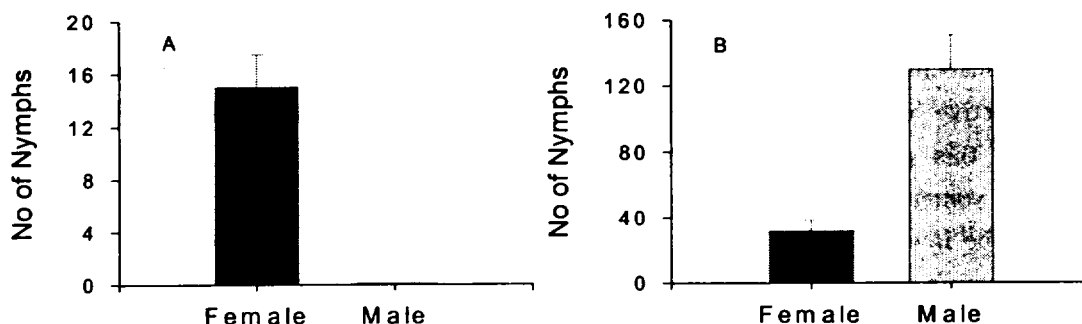


Fig. 5. The results of two-leaf choice test between male and female crawlers.

DISCUSSION

Unaspis yanonensis showed very different characteristics in morphology and settling behavior of its nymphs between males and females. It is important to distinguish female crawlers from male crawlers with a simple method such as color. It can be used in separated-rearing between females and males for the bioassay of pesticides and temperature-dependent development study. At present the distinction depends on individual experiences. Further studies such as color image analysis using digital signal are required to standardize the color pattern in the future.

The shell-like cover of scale insects is considered a protective strategy against their natural enemies. In the developmental process of many scale insects, the female cover develops a concentric ring around the nipple-shaped center with each molt (Kosztarab and Kozar, 1988). The female of *U. yanonensis* produced a thick protective cover from the 3rd nymphal instar (Fig. 3 ⑩). We conjecture that the integument of *U. yanonensis* nymphs as seen in Fig. 3 ⑤ & ⑩ is not a kind of the protective cover, although further investigations are needed for that. Consequently, we divide the development stage of *U. yanonensis* female into two stages of non-protective cover stage and protective cover stage according to the development of waxy scale cover. Such division is helpful for insecticide spraying. The acquiring the protective cover may prevent the penetration of insecticides into insect body. Thus, non-protective cover stage is the best stage to control this pest. In the future, field phenology of *U. yanonensis* based on the stage background will be investigated.

Unaspis yanonensis females go through two nymphal stages and reproduce in the third nymphal stage, consequently, the adult stage.

The males have five developmental stages: the first and second nymphal instars, prepupa, pupa, and alate imago (Kuwana, 1923). In the Fig. 3 ④, the exuviae of 1st (a) and 2nd (b) nymph are seen. Also, the exuviae of 1st nymph of male is seen in Fig. 4 ④ (a). The 2nd nymphal instars, prepupa, and pupa would have been developed in the white wax cover.

Unaspis yanonensis crawlers showed different settling behavior between males and females. Male crawlers settled down in clustered groups with nearly regular spacing each other. Also, they left the leaf where they were born. In general the crawlers of scale insects move around to find a suitable place to settle and can be spread about by wind, birds, or picking crews. These dispersal means can be applied to *U. yanonensis*. However, the destinations of such spread means are unpredictable place. Since male crawlers settled down in clustered group, their settling place is dependent on other individuals. So, accidental movement is not appropriate for *U. yanonensis* males. The settling behavior of *U. yanonensis* is an interesting study field and requires further researches.

The 1st generation female crawlers, which were born on overwintered leaves, settled down on spring leaves as well as on the overwintered leaves, while male crawlers were not found on spring leaves. All male crawlers took their position at the leaves where their mothers inhabited, consequently overwintered leaves. For the 2nd generation crawlers which were born on spring leaves or probably on overwintered leaves, the same dispersal pattern was observed with those of the 1st generation. A part of the 2nd female crawlers went away the leaf on which they were born, and moved to the summer leaves, while male crawlers did not. The dispersal strategy of the females is considered a survival adaptation for successful

reproduction on new nourishing leaves. The overwintered leaves infested with higher population of *U. yanonensis* dried and fell down during mid-season. The females appears to be selecting a good strategy for survival.

적 요

화살각지벌레 (*Unaspis yanonensis*)는 제주 감귤에서 심각한 해충중 하나이다. 이동성 약충기 (crawler nymph)에 만 분산할 수 있기 때문에 이들의 정착행동과 형태적 특징 변화에 대한 지식은 이 해충의 방제수단 및 실험설계를 용이하게 하기 위하여 중요하다. 화살각지벌레 부화약충의 체색은 암수 간 차이가 있었다. 대부분 암컷약충은 부화후 부터 정착초기까지 황색을 띄었고, 같은 기간동안에 수컷은 옅은 황색이었다. 암컷은 정착과 동시에 2개의 긴 가늘 실 모양 왁스 물질을 머리 끝에서 분비하였고, 보호껍질이 없는 두 약충발육단계를 경과하였다. 반면 수컷은 짧고 굵슬형의 조밀한 왁스물질을 등위에 생성하고 나서 결국 등 위에 세 줄의 흰 왁스물질을 형성하였다. 암컷의 영구적인 보호 껍질은 2령 약충 탈피각 끝으로부터 점차적으로 형성되었다. 이렇게 암컷의 발육단계는 보호껍질의 발달에 따라 미보호껍질 발육단계와 보호껍질 발육단계로 구분되었다. 또한 정착행동도 수컷과 암컷 간 매우 달랐다. 수컷은 일정한 규칙적 거리를 유지하면서 집단으로 정착하였다. 암컷은 집단으로 정착하지 않았으며 단독으로 또는 서로 접촉하여 또는 겹쳐서도 정착하였다. 부화약충 두 잎 간 선택 시험에서 암컷은 자유롭게 분산하여 그들이 태어난 잎뿐만 아니라 다른 쪽의 잎에도 정착하였다. 하지만 수컷은 그들의 어머니가 있는 잎에서 자리를 잡았다. 이러한 분산행동은 포장상태에서도 관찰되었다. 암컷은 월동된 잎에서 봄에 발생한 잎으로 그리고 봄 잎에서 여름에 발생한 잎으로 순차적으로 분산하였다.

검색어 : 화살각지벌레, 감귤, 정착행동, 분산, 체색

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