

Experimental and Applied Acarology

Effect of different ornamental pepper pollens on the development and reproduction of *Amblyseius swirskii* (Acari: Phytoseiidae) --Manuscript Draft--

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Corresponding Author:	Vivek Kumar, Ph.D. Mid-Florida Research and Education Center, University of Florida Apopka, Florida UNITED STATES
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Mid-Florida Research and Education Center, University of Florida
Corresponding Author's Secondary Institution:	
First Author:	Vivek Kumar, Ph.D.
First Author Secondary Information:	
Order of Authors:	Vivek Kumar, Ph.D. Vitalis W. Wekesa, Ph.D. Pasco B. Avery, Ph.D. Charles A. Powell, Ph.D. Cindy L. McKenzie, Ph.D. Lance S. Osborne, Ph.D.
Order of Authors Secondary Information:	
Abstract:	<p>Chilli thrips, <i>Scirtothrips dorsalis</i> Hood (Thysanoptera: Thripidae), a newly introduced pest in the United States is well known to cause significant economic damage on a variety of crops worldwide. In Florida, it has emerged as a key pest of ornamental and vegetable crops. Chemical control is still considered as a primary mode for its control. The rationale behind the current study was to assess if the phytoseiid mite, <i>Amblyseius swirskii</i> (Athias-Henriot), a commercially available predator of <i>S. dorsalis</i> can survive and reproduce by solely feeding on a variety of ornamental pepper pollens. In order to evaluate nutritional value and the possible impact on longevity, daily rate of oviposition and total egg deposition was assessed. <i>A. swirskii</i> was reared on pollen collected from four ornamental pepper varieties, viz. Red Missile, Masquarade, Black Pearl and Explosive Ember - potential banker plant candidates for ornamental nurseries. <i>A. swirskii</i> was able to survive, develop and oviposit on all the ornamental pepper pollen varieties tested and the two standard controls (commercially available olive pollen and <i>S. dorsalis</i>). No significant differences were observed among the different diet treatments for the following <i>A. swirskii</i> biological parameters: mean longevity (22.7-24.2 d), larvae to eggs (11.8 -12.6 d) and duration of adult stage (12.4-13.2 d). Amongst the pepper pollens, the highest daily rate of oviposition (~1.07 eggs/female/day) and total oviposition (~10.23 eggs/female) was recorded on the Red Missile variety. Results from this study can aid in the selection of suitable pepper varieties as candidate banker plants for establishment of <i>A. swirskii</i> in ornamental nurseries and can boost the management strategies being used against <i>S. dorsalis</i> and other pest species.</p>

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4 1 **Experimental and Applied Acarology**

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9 3 **Effect of different ornamental pepper pollens on the development and**
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11 4 **reproduction of *Amblyseius swirskii* (Acari: Phytoseiidae)**
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15
16 6 Vivek Kumar^{a*}, Vitalis W. Wekesa^b, Pasco B. Avery^c, Charles A. Powell^c, Cindy L. McKenzie^d,
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18 and Lance S. Osborne^a
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22
23 9 ^aDepartment of Entomology and Nematology, Mid-Florida Research and Education Center,
24
25 University of Florida, Apopka, FL, 32703, USA
26 10

27
28 11 ^bDepartment of Biological Science and Technology, Kenya Polytechnic University College,
29
30 (A constituent college of the University of Nairobi), P.O Box 52428-00200, Nairobi, KENYA.
31 12

32
33 13 ^cUniversity of Florida, Institute of Food and Agricultural Sciences, Indian River Research and
34
35 Education Center, 2199 South Rock Road, Fort Pierce, FL 34945, USA
36 14

37
38 15 ^dU. S. Horticultural Research Laboratory, USDA-ARS, Fort Pierce, FL, 34945, USA
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40

41 16
42
43 17 *Corresponding author: Vivek Kumar

44
45 18 vivekiari@ufl.edu

46
47 19 2725, South Binion Road

48
49 20 Mid-Florida Research and Education Center, University of Florida

50
51 21 Apopka, FL-32703

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53 22 Ph. 1-352-871-8088, Fax: 1-407-814-6186
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4 **25 Abstract**

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7 26 Chilli thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae), a newly introduced pest in
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10 27 the United States is well known to cause significant economic damage on a variety of crops
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12 28 worldwide. In Florida, it has emerged as a key pest of ornamental and vegetable crops.
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15 29 Chemical control is still considered as a primary mode for its control. The rationale behind the
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17 30 current study was to assess if the phytoseiid mite, *Amblyseius swirskii* (Athias-Henriot), a
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20 31 commercially available predator of *S. dorsalis* can survive and reproduce by solely feeding on a
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22 32 variety of ornamental pepper pollens. In order to evaluate nutritional value and the possible
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25 33 impact on longevity, daily rate of oviposition and total egg deposition was assessed. *A. swirskii*
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27 34 was reared on pollen collected from four ornamental pepper varieties, viz. Red Missile,
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30 35 Masquarade, Black Pearl and Explosive Ember - potential banker plant candidates for
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32 36 ornamental nurseries. *A. swirskii* was able to survive, develop and oviposit on all the ornamental
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35 37 pepper pollen varieties tested and the two standard controls (commercially available olive pollen
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37 38 and *S. dorsalis*). No significant differences were observed among the different diet treatments for
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40 39 the following *A. swirskii* biological parameters: mean longevity (22.7-24.2 d), larvae to eggs
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42 40 (11.8 -12.6 d) and duration of adult stage (12.4-13.2 d). Amongst the pepper pollens, the highest
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44 41 daily rate of oviposition (~1.07 eggs/female/day) and total oviposition (~10.23 eggs/female) was
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47 42 recorded on the Red Missile variety. Results from this study can aide in the selection of suitable
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50 43 pepper varieties as candidate banker plants for establishment of *A. swirskii* in ornamental
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52 44 nurseries and can boost the management strategies being used against *S. dorsalis* and other pest
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54 45 species.
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59 47 **Keywords:** Chilli thrips, banker plants, phytoseiid mites, biological control
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4 **48 Introduction**

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6 49 In last two decades, biological control has been practiced as an important component of pest
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9 50 management strategies. It is a method of suppressing arthropod pest populations using suitable
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11 51 natural enemies to gain an economic benefit resulting from the integration of various biological
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14 52 control approaches including classical, inundative and conservation (Gurr et al. 1998; Gurr and
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16 53 Wratten 1999). Banker plant systems are examples of an integrated biological control approach
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19 54 which involves combined aspects of augmentative and conservational biological control
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21 55 and habitat manipulation proposed as an efficient alternative to chemical based pest management
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24 56 techniques (Osborne and Barrett 2005; Frank 2010; Huang et al. 2011; Xiao et al. 2011). Success
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26 57 of biological control strategies depends upon several factors: (1) efficacy of natural enemies
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29 58 against the target pest(s), (2) high search ability of natural enemies, (3) seasonal synchronization
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31 59 with host, (4) adaptability to environmental variations and (5) continuous supply of food
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34 60 supporting survival and long-term establishment in the habitat. According to Gurr et al. (2000),
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36 61 success of biological control strategies can be valued by its affordability in bringing a pest
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39 62 population below the economic injury level, as well as the proportion of growers that adopt the
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41 63 technique. Installing banker plants in the agro-ecosystem can provide long-term suppression of
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44 64 pest populations by providing ecological infrastructures required to sustain a reproducing
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46 65 population of natural enemies (Frank 2010; Huang et al. 2011). Such a self-sustaining pest
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48 66 management system can increase reliability of biological control strategies and can reduce
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51 67 overall insecticide use. In addition to providing nutrient supplements in the form of nectar or
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53 68 pollen, important for the natural enemies survival in the absence of prey, banker plants can
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56 69 provide a modified microhabitat (domatia) which can protect them against adverse abiotic
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58 70 conditions, secondary enemies or from an insecticide application (Landis et al. 2000). The

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4 71 intentional provision of food and shelter via banker plants can maximize biocontrol potential of
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6 72 the released biological control agents and favor their survival, fecundity and longevity; thereby
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9 73 supporting the success of biological control agents overall.

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11 74 Chilli thrips *Scirtothrips dorsalis* Hood is a new invasive thrips species in the United
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14 75 States and an emerging pest of various vegetable, ornamental and tropical fruits in the region
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16 76 (Seal et al. 2006, 2010; Kumar et al. 2012). It is a polyphagous insect with more than 100
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19 77 reported hosts from 40 different families of plants worldwide (MacLeod and Collins 2006).
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21 78 Since its introduction in the United States in 2005, it has become established in 30 counties of
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24 79 Florida, 8 counties in Texas and with several reports of their invasion in Alabama, Georgia,
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26 80 Louisiana and New York (Kumar et al. 2011). Osborne (2009) recently reported that this pest is
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29 81 responsible for damaging more than 50 ornamental plant species in different parts of Florida.
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31 82 According to an economic analysis by Garrett (2004) on 28 potential hosts in the US, a 5% loss
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34 83 of these crops could lead to a loss value of \$3 billion to the USA economy. Thus, there is an
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36 84 urgent challenge for researchers to develop an efficient and long-term management strategy
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39 85 against this pest before it emerges as a serious control problem for vegetable and ornamental
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41 86 industries in the United States.

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44 87 In a previous study, the efficacy of two phytoseiid mites, *Amblyseius swirskii* (Athias-
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47 88 Henriot) and *Neoseiulus cucumeris* Oudemans, was evaluated (Arthurs et al. 2009) as potential
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49 89 biological control agents of *S. dorsalis*. *A. swirskii* was reported to provide significantly higher
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52 90 suppression of *S. dorsalis* populations compared to *N. cucumeris* and is being considered as a
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54 91 promising tool for their control. Although *A. swirskii* is an aggressive predator and can provide a
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57 92 significant reduction of *S. dorsalis* in a timely fashion, the survival and long-term establishment
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59 93 of the phytoseiid mites can be difficult in the absence of their prey. Scarcity of food may lead to

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94 intraguild predation resulting in a population crash of the phytoseiid mites, and the control
95 strategy would no longer be economical if multiple applications of the agents were required.
96 With the aim to develop a long-term management strategy for *S. dorsalis* using phytoseiid mites
97 by employing a banker plant based biological control strategy, we conducted a pilot study to
98 assess the effect of different ornamental pepper pollen on the life parameters of *A. swirskii*. The
99 current study was designed to determine whether *A. swirskii* can develop and reproduce when
100 feeding solely on an ornamental pepper pollen diet. Ornamental pepper cultivars were selected
101 because the original work was aimed to regulate *S. dorsalis* populations on rose nurseries and
102 gardens in the landscape, and the evaluation of different pollen was important for screening a
103 particular ornamental pepper banker plant variety.

Materials and Methods

Host plant and pollen collection

107 The study was conducted at the University of Florida's Indian River Research and Education
108 Center, Fort Pierce, FL (27.42N, 80.40W). The host plants for the study were grown from seed
109 as single plants in Fafard Pro-mix medium (Conrad Fafard, Inc., Agawam, MA) in plastic pots
110 (10.1 cm dia) placed into a homemade plastic screened cage (61 cm x 71 cm x 61 cm). Seeds of
111 the four different ornamental pepper (*Capsicum annum* L.) varieties (Masquerade (MA), Red
112 Missile (RM), Black Pearl (BP) and Explosive Ember (EE)) (Ball Horticultural Co., West
113 Chicago, IL, USA) were sown on different days to synchronize the germination times. Plants
114 were irrigated as needed (~3 times a week) and fertilized with 50 mL/pot of Peters Professional®
115 20-10-20 (325 ppm) (Scotts Co., Marysville, OH) once a week. Plants selected for the study
116 were healthy, young, vigorous, and free of arthropod pests. Two weeks after flowering, pollen

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4 117 was collected from flowers of each variety by tapping the pollen into small plastics ampoules.
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6 118 The collected pollen was dried in an oven for two days at 37°C before being stored in a
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9 119 refrigerator at 4°C.
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14 121 **Stock colony of *A. swirskii***

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16 122 *A. swirskii* population used in the study was collected from a colony which was initially
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18 123 established at Mid-Florida Research and Education Center, Apopka, FL (28.63N, 81.55W) in
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20 124 2009 from a culture of SWIRSKI-MITE™ (Koppert Biological Systems., Inc. USA Ltd, Howell,
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22 125 MI). The mites were raised following a modified protocol of Carrillo et al. (2010) in plastic trays
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24 126 (14 x 14 cm) filled with water; culture arena consisted of a waxed colored paper (5.5 x 5.5 cm)
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26 127 grooved by a wire mesh (1 mm²) placed on top of 3 stacked cotton pads (75 mm dia) with a few
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28 128 threads of cotton placed on top of the waxed paper simulating leaf trichomes (to facilitate egg
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30 129 oviposition of mites). Small dried apricots infested with all stages of *Carpoglyphus lactis* (Acari:
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32 130 Acaridae) were supplied to *A. swirskii* on plastic arenas as a food source. In order to produce
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34 131 mites of the same age, gravid female *A. swirskii* were kept individually in small Falcon® Petri
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36 132 dishes (4 cm dia) for 12-14 h to oviposit on leaf disks. The eggs were collected and incubated at
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38 133 25°C. The mites that emerged were considered to be of the same age or at least formed cohorts
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40 134 with a maximum age variation of less than 8 h.
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50 136 **Feeding experiment with *A. swirskii***

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53 137 The experimental arena consisted of a small Falcon® Petri dish (4 cm dia) lined with a moist
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55 138 cotton layer and sealed with plastic food wrap. Leaf discs (3 cm dia) of each pepper variety were
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58 139 punched at the center of the leaf blade beginning at the intersection between the petiole and the
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4 140 edge of the leaf with a cork borer and placed on the cotton. About ~13-15 mg of pollen was
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7 141 placed on plastic cover slips and the cover slips were replaced every three days to avoid mold
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9 142 growth. The cover slips were placed at the center of each leaf disk. The ornamental pepper
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12 143 treatments (pollen) evaluated in the study was RM, MA, EE and BP. For comparison, a
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14 144 commercial pollen source from an olive plant (OC) (Pollen Collection and Sales, Inc. Lemon
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16 145 Cove, CA) and *S. dorsalis* (SD) larvae were used as controls; these were put on MA leaves as
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19 146 this variety was more leafy than other ornamental pepper varieties. A single gravid female mite
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21 147 was gently removed from one of the rearing units using a moistened camel hair brush, and
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24 148 transferred to the center of the leaf disk and allowed to walk off the brush onto the cover slip.
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26 149 Fifteen replicate bioassays were placed on a tray for each treatment and placed on a shelf in the
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29 150 growth chamber held at 25 ± 1 °C, $75 \pm 5\%$ RH and a 14 h L: 10 h D photoperiod. Egg deposition
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31 151 and hatching of *A. swirskii* for each treatment was assessed using a binocular microscope (40X)
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34 152 every 24 h for two weeks and the study was repeated once.
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38 154 **Statistical Analysis**

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41 155 Data obtained on *A. swirskii* (egg numbers, motile stages and developmental period) during the
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43 156 study were analyzed independently using the Statistical Analysis System (SAS Institute Inc.
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45 157 2003). Data were transformed by the square-root of $(X + 0.25)$ to stabilize error variance before
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48 158 analyses. The transformed data were then analyzed using one-way analysis of variance
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51 159 (ANOVA). Differences among the treatment means were tested using the Tukey's honestly
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53 160 significant difference (HSD) at $\alpha = 0.05$. The non-transformed means are presented in the table
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55 161 and figure.
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4 **163 Results**

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6 164 Data from the two experiments were pooled together because there was no treatment-by-
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9 165 experiment interaction observed. *A. swirskii* was able to survive, develop and oviposit after
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11 166 feeding on either pollen or *S. dorsalis* (SD) only (Table 1). Longevity of *A. swirskii* varied
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14 167 between 22.7-24.2 d when fed on different treatments (SD = 21-26 d; OC = 21-25 d; RM = 21-
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16 168 25 d; MA = 21-25 d; BP = 20-25 d; EE = 22-26 d), however no significant differences ($F = 2.38$;
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19 169 $df = 5, 174$; $P = 0.0603$) were observed among the means of different treatments for the two
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21 170 replicated experiments. There were no significant differences observed in other life cycle
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24 171 parameters including larvae to first egg stage ~11.5-13.2 d ($F = 1.57$; $df = 5, 174$; $P = 0.1707$)
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26 172 and duration of adult stage ~12.1-13.5 d ($F = 2.21$; $df = 5, 174$; $P = 0.0551$) when phytoseiid
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29 173 mites fed on different food sources. However, the type of diet did influence reproductive
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31 174 potential of *A. swirskii*. A significantly higher number of *A. swirskii* eggs was recorded when
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33 175 mites were offered *S. dorsalis* as a food source (7-22 eggs/adult) compared to pollen treatments
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36 176 ($F = 19.37$; $df = 5, 174$; $P < .0001$), followed by RM (4-18 eggs/adult) and OC pollen (3-15
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38 177 eggs/adult), respectively. The daily oviposition rate of *A. swirskii* fed on *S. dorsalis* was
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41 178 significantly higher than MA, EE and BP, but not from RM and OC ($F = 3.60$; $df = 5, 174$; $P =$
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43 179 0.0040) (Figure 1).
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50 **181 Discussion**

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52 182 Biological control is an important component of integrated pest management (IPM) for any
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55 183 target pest species. Combining an inundative approach along with conservational biological
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57 184 control can aid the long term survival of biocontrol agents and result in achieving the goal of an
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59 185 IPM program- economically efficient strategy; which in turn can increase the acceptability of the
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186 management strategy. According to Osborne and Barrett (2005), the establishment of biological
187 control agents in a new environment is a critical and difficult process. Thus it is essential to
188 develop a banker plant based management strategy which is a self contained tool that is needed
189 to meet the growing demand of sustainable agriculture and help facilitate the establishment of
190 biological control agents during this critical stage. The phytoseiid mite, *A. swirskii* is a generalist
191 predator, both nymphal and adult stages are voracious feeders and well known for their
192 biocontrol potential against whiteflies and many different thrips species in greenhouse and
193 nursery crops (Nomikou et al. 2001; Van Houten et al. 2005; Messelink et al. 2006). However,
194 there is limited information available about their performance in regulating *S. dorsalis* in
195 ornamental nurseries. Results from the current study on biological and reproductive parameters
196 of *A. swirskii* fed on pollen from different ornamental pepper varieties points to the fact that the
197 mite species can successfully survive and establish in nurseries exclusively feeding on pollen
198 produced by the plant. Although, *A. swirskii* was able to survive and reproduce on all the pollen
199 treatments, the highest mean longevity, daily rate of oviposition and total number of eggs was
200 recorded on Red Missile, suggesting that this variety must be subjected to further screening as a
201 potential ornamental banker plant for IPM of *S. dorsalis*. Because implementation and
202 maintenance of the banker plant is easy and economical, and phytoseiid mites are facultative
203 feeders, *A. swirskii* can establish and provide suppression of multiple pests in ornamental
204 nurseries.

205 Apart from producing pollen or nectar as a nutritional supplement, another factor which
206 is critical for selecting the best candidate as a banker plant is the presence of tuft domatia
207 (domatia with non-glandular trichomes in the vein axils of leaves) (O' Dowd and Wilson 1991;
208 Walter 1996). There are several reports indicating that the presence of tuft domatia can support

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209 the survival and establishment of beneficial mites in a number of ways, such as providing a
210 breeding and development site, protection against adverse climatic condition, protection against
211 natural enemies, etc. (O' Dowd and Wilson 1991; Walter 1996; Roda et al. 2000; Romero and
212 Benson 2005). Lounghner et al. (2008, 2010) showed that the presence of tuft domatia was
213 positively associated with an increased abundance of phytoseiid mites. Thus, presence or absence
214 of tuft domatia on the selected plant variety can be a crucial factor governing the population
215 increase of *A. swirskii* in the new banker plant habitat. Our future studies will focus on
216 evaluating other parameters of a successful banker plant including presence of tuft domatia, and
217 the preference of *A. swirskii* among the four pepper varieties studied here in the presence and
218 absence of prey and pollen.

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302 **Figure caption**

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304 **Figure 1.** Daily oviposition of *Amblyseius swirskii* females fed on either Red Missile,
305 Masquarade, Black Pearl, Explosive Ember, olive pollen or *Scirtothrips dorsalis* (25 ± 1 °C, $75 \pm$
306 5% RH and a 14 h L: 10 h D). Error bars represent the standard error of the mean (Tukey's HSD,
307 $P < 0.05$).

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Figure
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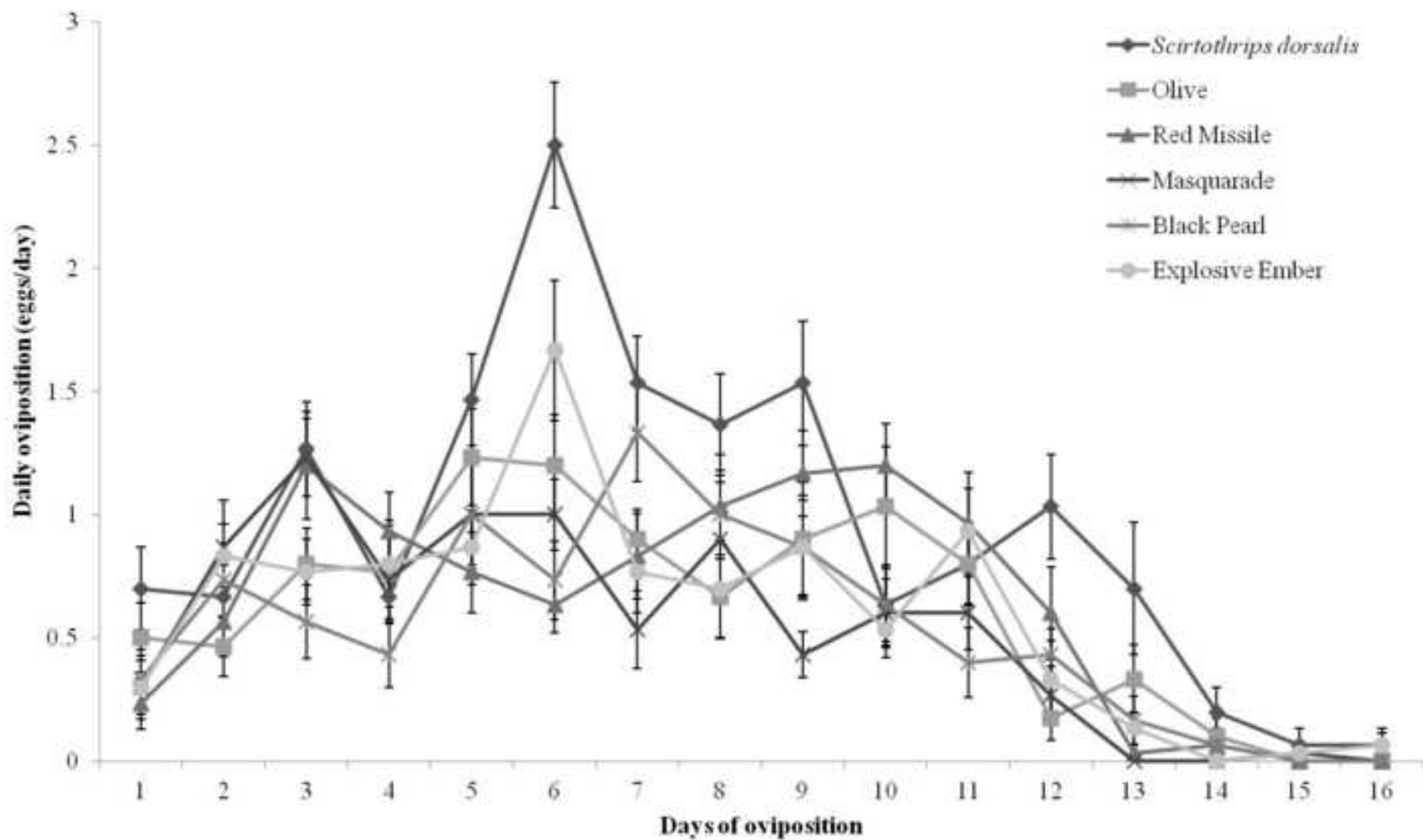


Table 1. Biological parameters (development and reproduction) of *Amblyseius swirskii* fed on six different food sources (five pollen types; OC, RM, MA, EE, BP and thrips SD at 25 ± 1 °C, $75 \pm 5\%$ RH under a 14 h L: 10 h D photoperiod)

	<i>S. dorsalis</i> (SD)	Olive (OC)	Red Missile (RM)	Masquarade (MA)	Explosive Ember (EE)	Black Pearl (BP)
Longevity (days)	23.13 ± 0.25a	22.96 ± 0.26a	23.63 ± 0.21a	23.26 ± 0.26a	23.76 ± 0.24a	22.83 ± 0.21a
Larvae to eggs (days)	11.80 ± 0.15a	12.53 ± 0.38a	12.53 ± 0.18a	12.10 ± 0.14a	12.36 ± 0.30a	12.56 ± 0.24a
Duration of adult stage (days)	13.03 ± 0.24a	12.46 ± 0.26a	13.10 ± 0.21a	12.50 ± 0.26a	13.16 ± 0.20a	12.43 ± 0.26a
Oviposition period (days)	11.56 ± 0.33a	9.70 ± 0.51ab	9.53 ± 0.29ab	8.56 ± 0.55b	9.70 ± 0.44ab	9.20 ± 0.48b
Post oviposition period (days)	0.73 ± 0.21c	1.20 ± 0.22bc	2.03 ± 0.29ab	2.96 ± 0.53a	2.10 ± 0.29ab	1.66 ± 0.28abc
Total number of eggs	15.20 ± 0.66a	9.86 ± 0.54b	10.23 ± 0.56b	8.50 ± 0.51b	9.60 ± 0.55b	8.70 ± 0.56b
Daily rate of oviposition (eggs/day)	1.33 ± 0.06 a	1.10 ± 0.08ab	1.07 ± 0.04ab	1.05 ± 0.05b	1.01 ± 0.05b	1.02 ± 0.08b

Means within a row followed by the same letter are not significantly different ($P \geq 0.05$, Tukey's HSD).