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# Preference, population development, and molecular characteristics of *Spodoptera exigua* (Lepidoptera: Noctuidae) on shallot cultivars: A field trial scale

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**Abstract.** *Supartha IW, Susila IW, Sumiartha IK, Rauf A, Cruz LBDC, Yudha IKW, Utama IWEK, Wiradana PA. 2022. Preference, population development, and molecular characteristics of* Spodoptera exigua *(Lepidoptera: Noctuidae) on shallot cultivars: A field trial scale. Biodiversitas 23: 783-792.* The beet armyworm, *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae), is a polyphagous insect that is the main pest and is responsible for the decline in shallot production in Indonesia. This study aims to (i) analyze preferences; (ii) colonization time; (iii) population development; (iv) attack of armyworms according to plant phenology in the shallot cultivars; and (v) identification species of armyworms. The research was conducted in Bangli Village, Tabanan Regency, Bali Province, Indonesia. The research design used was a randomized block design with the treatment of five shallot cultivars and five replications. Molecular analysis of armyworm larvae was carried out using the Polymerase Chain Reaction (PCR) method using forward Lep_F1 and reverse Lep_R1 primers at the Cytochrome Oxidase Subunit I (COI) gene. Our results indicate that the period of adult pest infestation has been seen since the emergence of plants, namely 7 days after planting (dap). The colonization period begins at 21 (dap), when plants and larvae begin to grow. The development of the egg and larva population was seen when the plants totaled 21 (dap) (for eggs) and 28 dap (for larvae). The most preferred shallot cultivars are "Bali Karet" and "Bima Brebes". Armyworm larvae, including *S. exigua*, were identified as related to the same group as isolate larvae from China, India, Pakistan, Thailand, and Japan. These findings indicated that *S. exigua* preferred the two cultivars of shallots in the fields and emerged at 21 dap. This shows that insect management activities must be carried out systematically both when the shallots plants are young, pre- and post-harvest. Control with natural enemies can also be done for further research.

**Keywords:** Agriculture, beet armyworm, crop protection, invasive pest, plant phenology

## INTRODUCTION

Shallots are a strategic vegetable product that is now a leading agricultural commodity because it has a high contribution to economic development in Indonesia (Pratiwi et al. 2019). The shallot commodity has long been cultivated intensively by farmers in Indonesia because it is able to provide job opportunities and a decent source of income for farming communities (Van den Brink and Basuki 2012; Rahayu et al. 2019). Several places in Indonesia, including North Sumatra, West Sumatra, West Java, Central Java, Yogyakarta, East Java, Bali, West Nusa Tenggara, and South Sulawesi, produce more than 1000 hectares of shallots every year (Potter 2001; Udiarto et al. 2005). These regions are predicted to contribute around 95.8% of the shallot crop, with Java Province supplying over 75% output in 2013 (Susanawati et al. 2018; Aldillah 2020). As a result, farmers are particularly interested in cultivating shallots, and farming operations are expanding fast across Indonesia (Sutardi and Purwaningsih 2018; Swastika et al. 2019).

One of the most significant challenges for shallot producers is the presence of pests and diseases, both of which have broad and varied effects (Ujiyani et al. 2019). Armyworms, which have a high malignancy and have highly negative impacts, are still a problem for farmers (Marsadi et al. 2017). The beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), is worldwide due to its extensive distribution (Ueno 2015). According to the report, the existence of this pest is recognized as a classic pest in Indonesia, causing shallot crop loss in the lowlands of Java Province (Rauf 1999). Under some situations, beet armyworms are also a risk to highland shallot production.

The findings of our field monitoring at one of the shallot planting centers in Kedisan Village, Kintamani District, Bangli Regency (1000 masl), Bali Province, showed that armyworms are highly abundant, causing concern among local farmers. Farmers are interested in investing control expenses (through pesticides) to avoid these pests in order to save money on shallot output. Under severe assault situations, farmers may have to spend high control expenses to purchase synthetic pesticides that are sprayed on a regular basis every 2-3 days. Chemical control might cost between 30 and 50% of the entire cost. Unwise pesticide usage is not only financially damaging to farmers, but it also has a negative effect on the environment and human health (Murphy and Lasalle 1999; Mohanty et al. 2013; Susila et al. 2021).

The Indonesian government has designed an ecologically friendly pest management method to decrease the negative effect of using synthetic pesticides to control pests and illnesses in agricultural crops. Ecological pest management, commonly known as "green pest control," is a safe and effective integrated pest management strategy that has little or no impact on the environment or consumers (Srivani 2019). Integrated pest management (IPM) is an important component of any pest control program that aims to reduce pesticide application (Alam et al. 2016). Based on this, pest treatment required a responsible strategy that includes comprehensive and detailed pest identification, a systematic approach to identifying and discussing invasion sites and attractants, effective prevention and control, and long-term management (US EPA 2011). Understanding biological and ecological information about the target pest is essential both in the field and in the laboratory to simplify the deployment of pest management techniques and technology (Wahyuni et al. 2017; Supartha et al. 2020). Until recently, information on population growth and insect invasions in the highlands have been few. Furthermore, farmers are sometimes confused when it comes to obtaining pest-resistant types, particularly during the dry season. Based on this, our study aimed to analyze and comprehend ecological information on population growth and armyworm attack on several cultivars of shallot in the highlands by (1) analyzing the time and source of adult invasion into shallots; (2) armyworm colonization on shallot grown in the field; (3) analyzing the development of armyworms according to plant phenology; (4) analyzing the development of beet armyworm attacks according to plant phenology in the highlands; and (5) molecular characteristics of beet armyworm in this study.

## MATERIALS AND METHODS

### Study area

This research took place from April to September 2021 and was carried out on two scales, namely the field scale and the laboratory scale. The study was carried out at Bangli Village (1000 masl) in Baturiti District, Tabanan Regency, Bali Province, Indonesia. Because it is a shallot center area, the place was selected. The Integrated Pest Management Laboratory (IPMLab), Faculty of Agriculture, Udayana University, conducted a laboratory study on the production of eggs and larvae as well as parasitoid eggs and larvae related with *S. exigua* in the field. *S. exigua* preference in several shallot cultivars was also investigated in the laboratory.

### Research design and procedure

This study utilized a randomized block design with five replications of five (5) shallot *(Allium cepa)* cultivars, namely Bima Brebes (V1), Bauji (V2), Tajuk (V3), Probo (V4), and Bali Karet (V5) (Figure 1). This experiment was carried out in Bangli Village, Baturiti District, Tabanan Regency (1000 masl). The used land area is 2000 $m^2$. Raised beds measuring 1.5×7 m are constructed in this region. Each bed is separated by a 0.5 m wide by 0.4 m high ditch. The total number of beds utilized is 100. The spacing utilized was 19×23 cm, resulting in around 196 clumps in each bed. Unless an insecticide was administered, plant maintenance involves watering, cultivating, and fertilizing according to local farmer practices.

### Time of invasion and colonization of *Spodoptera exigua* on shallot cropping

The invasion time is the time when adult males are seen entering in the shallot plantings through the Exi pheromone traps placed diagonally on the 5 treatment plots. Exi Pheromone is a sex pheromone attractant that is particularly developed to attract adult male adults *(S. exigua)*. Meanwhile, the invasion of adult female *S. exigua* was seen by egg groups put on the plant 7 days after planting (dap). Meanwhile, the period of colonization is the beginning of a pest colony, which occurs from the beginning of the egg group hatching, instar-1 larvae that begin attacking the plant.

Observation of the number of trapped adults was carried out every day on traps that came to the shallot fields by counting the number of adults trapped in the 36 trap boxes. Colonization was also carried out by the absolute method starting a day after planting (1 dap) to see the initiation of adult females laying eggs on plants. Observation of *S. exigua* eggs was carried out in conjunction with the installation of Exi traps in shallot cultivation in the field. Observation of the number of egg groups was carried out for a week from the time the plants were two weeks old (early shoots appeared on the ground) to two weeks before harvest. Meanwhile, the observation of the colonization process was carried out through larvae that hatched from egg groups from 2 dap - 2 weeks before harvest.

### Preference and adaptation of *Spodoptera exigua* on shallot cultivars

The number of larval populations that survived in each shallot cultivar was used to determine *S. exigua* preference and adaptation to shallot cultivars. Larval population assessments were carried out every week starting the 2nd week after planting to 2 weeks before harvest. A sampling of larvae using the U-shape method of 25 plant clumps per sample point or 125 plants per observation plot with an area of 2×1.5×20 m.

### Population development of *Spodoptera exigua*

Observation of larval populations was carried out using the same as the previous method from Sunari et al. (2022), namely through a sampling of larvae using the U-shape method of 25 plants per sample point or 125 clumps per observation plot with an area of 2×1.5×20 m (Figure 1). Observations of the larvae population were carried out every week, starting the 2nd week after planting until 2 weeks before harvesting.

**The development of *Spodoptera exigua* population according to plant phenology of shallot cultivar**

Observation of the number of infected leaves was carried out by counting the total number of leaves and the number of infected leaves per plant. Observations were made every week starting the second week after planting up to two weeks before harvesting. Samples of infected plants were taken using the U-shape method, as many as 10 plants per plot on an observation plot measuring 1.5×20 m. The percentage of attacks is calculated using the formula:

$$P = \frac{a}{b} \times 100\%$$

Where, $P$ is attack percentage (%), $a$ is number of leaves attacked, and $b$ is the total number of plant leaves.

**Molecular identifications for *Spodoptera exigua* larvae**

*DNA extraction*

DNA extraction was performed using the procedures described by Hamid et al. (2018) and Herlinda et al. (2021). In summary, *S. exigua* larvae discovered in the study area were preserved in 70% alcohol and kept in a -20°C freezer until the material was needed for isolation. The sample must be isolated, collected, and dried for 30 minutes on a tissue. The larvae were then immersed in hot water at 85°C for 30 minutes until they became moderately yellowish in color. Following that, the places in the two abdomen portions were sliced and placed into a 1.5 µL tube. Proteinase K in the amount of 5 µL was added and crushed until crushed. The crushed material was dissolved in 300 µL of TNES buffer containing 1 M Tris HCl (pH 7.5). 5 M NaCl, 0.5 M EDTA, ddH2O, and 20% SDS), homogenized and incubated at 60°C for 3 hours. Following the completion of the incubation period, 85 µL of 5 M NaCl was added and centrifuged for 10 minutes at 14000 rpm. The supernatant was collected in large quantities (up to 400 µL) and placed in a fresh tube with isopropanol, up to 60% of the volume of the supernatant taken. After that, place it in the freezer for 20 minutes. Centrifugation at 14000 rpm for another 5 minutes. The supernatant was removed, and 500 µL of cold 70% alcohol was added before centrifuging for 15 minutes at 14000 rpm. The supernatant was removed once again and dried for 24 hours at room temperature. Following drying, 20 µL of TE buffer was added (1st Base, Malaysia). Before use, DNA suspensions were kept at -20°C.

*DNA amplification*

The Cytochrome Oxidase Subunit I (COI) region was amplified using forward primer Lep F1 (5'-ATT CAA CCA ATC ATA AAG ATAT-3') and reverse primer Lep R1 (5'-TAA ACT TCT GGA TGT CCA AAAA-3'). Hebert et al. (2003) and Herlinda et al. (2021) followed the amplification process. In summary, each PCR included 5 µL of pH 8.3 PCR buffer (10 Mm Tris-HCl, pH 8.3; 1.5 Mm MgCl2; and 50 Mm KCl; 0.01% NP-40), 35 mL of distilled water, 200 mM dNTP, 1 unit of Taq Polymerase, 0.3 M primer, and 1-4 µL of DNA template. PCR was performed in steps, with one cycle for 1 minute at 94°C, five cycles for 1 minute at 94°C, 1.5 minutes at 45°C, 1.5 minutes at 72°C, 35 cycles at 1 minute at 94°C, 1.5 minutes at 50°C, and 1 minute at 72°C, and the last cycle for 5 minutes at 72°C. The PCR reactions were then electrophoresed on a % agarose gel using 1 µL of Ethidium Bromide (EtBr: 10 mg/mL/20 mL agarose) for 70 minutes at 55 V. A UV transilluminator was used to visualize the information (UVP, USA).

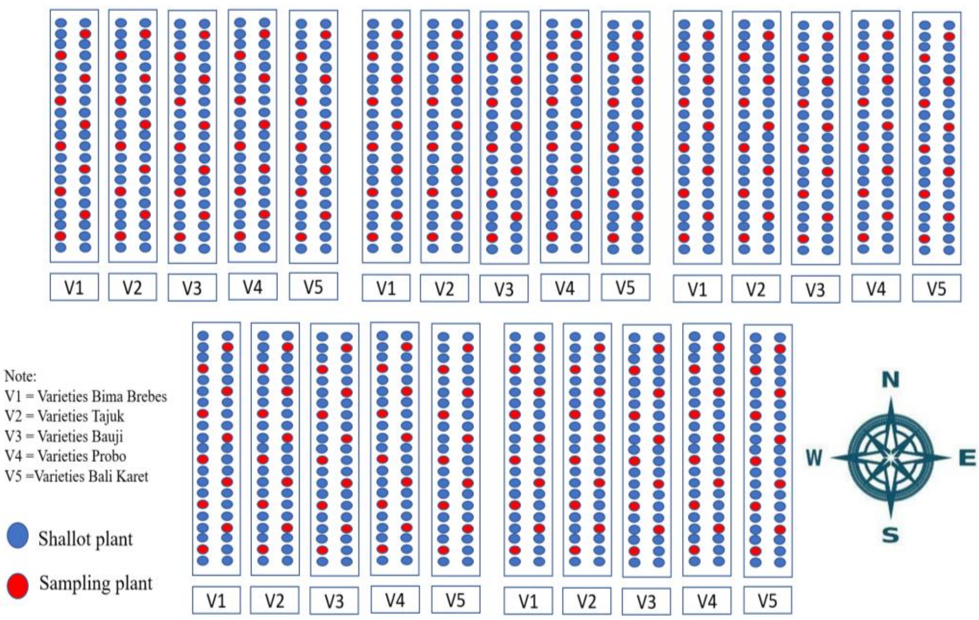


**Figure 1.** Sampling method in this study

*Sequencing and phylogenetic trees*

The PCR results obtained were continued with the sequencing process at 1st Base Malaysia. Sequencing results were read at the PT. Genetika Science Indonesia. The results of the base sequences of the two samples were analyzed in sequence to determine the difference in the base content of the protein using the Bioedit Version 7.0.5.3 application. The results of the analysis were then submitted to the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine the similarities and possible identities of the samples. MEGA application version 6.06 for Windows was used to calculate genetic distance. Then, the COI gene phylogenetic analysis was carried out using the Neighbor-Joining (NJ) method with a 1000 bootstrap (Tamura-Nei model). The reference strain used in this study was obtained from the NCBI program (https://www.ncbi.nlm.nih.gov/).

**Data analysis**

The collected data were tabulated in Microsoft Excel 2019 (Microsoft, USA) and analyzed using SPSS 23.0 (IBM, USA) software to determine the differences in each treatment using One Way ANOVA, followed by the Duncan multiple range test (DMRT) with a confidence interval of 5%. The analysis result data is displayed in the form of tables and figures.

## RESULTS AND DISCUSSION

### Time of invasion and colonization of *Spodoptera exigua*

The findings demonstrated that adults of *S. exigua* was found to enter some of the newly grown shallot plants, or 7 days after planting (dap). The existence of adults in plants may be physically seen in the field. In this research, the presence of armyworms may be determined by examining the spawning process around the shallot plants. The amount of eggs deposited and distributed across the leaves of the plants indicates the large population of *S. exigua* adults that attacked shallot plants in the field.

The existence of adult insect populations is also checked in the field by installing Exi pheromone traps. However, this trap can only catch mature males. As a result, the number of pests caught cannot be utilized to predict the number of eggs deposited in the field. Nonetheless, these captures may be utilized to provide an early indicator of the existence of *S. exigua* in study site. Our findings indicate that the highest invasion direction supports the development of mature plants at planting locations in Northeast 1 and Southeast 2 (Figure 2).

Figure 2 shows that the fluctuation of the adult population of *S. exigua* that occurred in plantations was originally relatively low until 6-8 weeks after planting. However, it then soared 70 days after planting. The colonization of eggs, larvae, and pupae in the shallot crop is regarded to be a major contributor to the increased prevalence of adults in the shallot crop. The colonization process started with the laying of the first egg in the shallot plant, which was seen in the second week following planting. While the first larval birth was recorded in the third week following planting. The process of colonization and recolonization as measured by the presence of eggs, larvae, and adults in the field is increasing according to plant phenology.

Because of its exceptional adaptability, *S. exigua* has expanded to effectively exploit numerous shallot cultivars. Marsadi et al. (2017) discovered that *S. exigua* has invaded shallot plants in Songan Village, Kintamani, Bangli. In the field, *S. exigua* was detected in practically all types of Batu Ijo and Bima Brebes. The appearance of mature males in the field, on the other hand, occurred towards the beginning of the growing season (7-21 dap), which happened in Batu Ijo but afterwards shifted to the Bima Brebes variety. The distance between the invasion source and the planting place has a significant impact on the invasion process. Because of the geographical extent of the field, it can be expected that the further distant the source of the invasion is, the lower the invasion rate may even be (Golikhajeh et al. 2016). Physical elements such as light, wind, climate, and rainfall have a significant impact on the invasion process of adult *S. exigua* (Marchioro and Foerster 2016; Tian et al. 2017; Skendžić et al. 2021). Based on the route of invasion in the field, the information gained may be used to choose the optimal pest management approach for *S. exigua*.

### Preference and adaptation of *Spodoptera exigua* on shallot cultivars

The population density of larvae living in each shallot plant cultivar was used to determine *S. exigua* preference and adaptation to various shallot cultivars. The results of the observation indicate that the preferences and adaptation of the larvae were significantly different (P <0.05) to the cultivar of shallot in the field. The most chosen cultivar is the Bali Karet, while the least chosen cultivar is the Probo (Table 1).

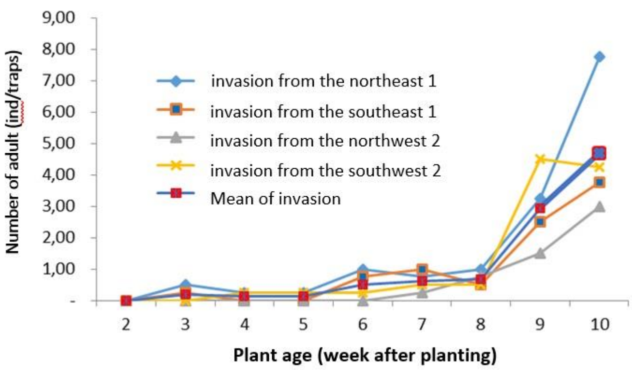


**Figure 2.** The number of adult *Spodoptera exigua* males adult monitoring using Exi Pheromones trap based on the direction of invasion in the field

**Table 1.** Preference and adaptation of *Spodoptera exigua* to shallot cultivars in this study

| Cultivars | Day After Planting (DAP) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 | 70 | 78 | 82 |
| | Number of larvae | | | | | | | | | | |
| Bima Brebes | 0 | 2.6 ab | 5.6 a | 10.4 a | 17.4 ab | 34.4 a | 31.2 a | 31.0 a | 27.2 b | 25.4 ab | 28.8 ab |
| Bauji | 0 | 0.4 b | 6.6 a | 5.8 b | 7.0 b | 33.6 a | 24.2 ab | 24.4 ab | 19.0 b | 18.4 bc | 24.0 ab |
| Tajuk | 0 | 1.2 ab | 5.8 a | 10.0 a | 12.0 ab | 23.8 a | 23.2 ab | 22.6 ab | 22.4 b | 15.2 c | 15.4 b |
| Probo | 0 | 0.4 b | 2.8 b | 5.0 b | 5.4 b | 9.0 b | 19.0 b | 23.4 b | 22.0 b | 26.0 ab | 26.4 ab |
| Bali Karet | 0 | 3.8 a | 8.8 a | 11.6 a | 33.0 a | 33.0 a | 36.0 a | 33.6 a | 40.8 a | 32.0 a | 34.8 a |

Note: Different letters in the same column indicate significant differences between cultivars ($p < 0.05$).

The population rate of *S. exigua* in the field is substantially influenced by host plants (Karimi-Malati et al. 2014). *Spodoptera exigua* may survive and spread to cultivated host regions by using a variety of host plants, including okra *(Abelmoschus esculentus)*, onion *(Allium cepa)*, leek *(A. cepa* var. aggregatum*)*, Welsh onion *(A. fistulosum)*, and garlic *(A. sativum)*. Armyworms are able to invade spinach *(Spinacia oleracea)*, radish *(Raphanus raphanistrum* subsp. *Sativus)*, cabbage *(Brassica oleracea)*, celery *(Apium graveolens)*, asparagus *(Asparagus officinalis)*, sugar beets *(Beta vulgaris)*, broccoli *(Brassica oleracea)*, chili peppers *(Capsicum* spp.), chrysanthemums *(Chrysanthemum indicum)*, oranges *(Citrus* spp.*)*, melons *Cucumis melo)*, carrots *(Daucus carota)*, strawberries *(Fragaria* sp.*)*, soybeans *(Glycine max)*, and cotton *(Gossypium* sp.*)*, in addition to onions (Zhang et al. 2011). According to our data, the greatest direction of invasion in the field occurred during shallot planting in the Northeast 1 and Southeast 2 directions. We assume this was related to the source of the mature *S. exigua* invasion, which used other crops cultivated in that direction, such as radish and cabbage. Preferences and adaptations of *S. exigua* larvae were evident from the 3rd week to the 10th week. After that, the preferences and adaptations strengthened in the Probo cultivar. This is because when the other plants had run out of leaves (dried), the Probo still showed a green color. These conditions are supported by the Probo cultivar characteristics that have a number of saplings and leaves in the lot, while Bali Karet has fewer (Figure 3).

The characteristics of the Bali Karet cultivar had more tillers and the number of real leaves was significantly ($P<0.05$) in the 2nd and 3rd weeks (Table 1) more attractive and invited more adults to come and lay eggs than other cultivars. This condition was seen in the population of *S. exigua* larvae, which increased significantly since the 3rd week.

Bali Karet showed as the cultivar most preferred by *S. exigua* in the field, as measured by the number of *S. exigua* populations and plant phenology. Meanwhile, the Brojo and Bauji types were more effective in preventing *S. exigua* recolonization. This incidence was highly suspected owing to the effect of the Bali Karet cultivar's features, which had more tillers and leaves in the early phases of development (14-21 dap), resulting in more appealing caterpillars to come and lay eggs. Determining this choice is critical since the host plant influences the creation, growth, survival, and fecundity of herbivorous insects (Awmack and Leather 2002). This is also attributable to the host plant quality components, such as carbon, nitrogen, and defense metabolites, which influence the potency and survival of herbivorous insects (Kant et al. 2015). Of course, this varies depending on the host plant environmental conditions (Moreira et al. 2018; Erb and Kliebenstein 2020). Another study found that *S. exigua* larvae favored cabbage leaves over shallots, chiles, long beans, and ladyfinger (Aziz and Azirun, 2006).


**Figure 3.** The cultivars of shallots tested in this study. 1. Bima Brebes; 2. Tajuk; 3. Bauji; 4. Probo; 5. Bali Karet

### The development of *Spodoptera exigua* population according to phenology of shallot cultivars

The development of the armyworm population is influenced by the cultivars of shallots in the field. The effect of these cultivars seemed from 28 (dap), 42 (dap), and until pre-harvest, which was 82 (dap). The crop that most strongly suppressed larval populations at the start of the attack were Probo cultivar, followed by Bauji, Tajuk, Bima Brebes and Bali Karet (Table 2). These conditions have implications for the average population of armyworms in shallot plants, which is at its lowest 63 (dap) in the Probo variety. However, at the time of the crop before harvest, the age of 82 (dap), the lowest density is in the Tajuk because the condition of the leaves is dry.

In the context of their life cycle and suitability to host plants, population growth and insect survival in various plant species/cultivars may be studied (Hong et al. 2019; Ali et al. 2021). The rapid rate of population growth over a short period of time may be linked to measures of the nutritional quality of the host plant (Cherif and Verheggen 2019). As a result, the increased population growth and adaptability of *S. exigua* may be a reflection of this host plant cultivars low nutritional quality (Golikhajeh et al. 2017). The shallot variety is regarded as an excellent host, as shown by significant population growth based on the phenology of the host plant, particularly the Bali Karet variety. Plant phenology is the foundation and is considered essential for understanding the relation between plants and pest populations (Elmendorf et al. 2016). This knowledge is very beneficial in developing more efficient and cost-effective pest control solutions (Kulkarni et al. 2019).

### The development of *Spodoptera exigua* attacks according to phenology of shallot cultivars

As indicated by the growth of the egg and larval population of *S. exigua*, the development of armyworm attacks in the field was also strongly influenced by the shallot plant variety (Table 3). Interestingly, from 49 (dap) to pre-harvest or 82 (dap), the impact of this cultivar was significant.

Bali Karet and Bima Brebes are the plants most susceptible to armyworm invasion in the early stages. At 49 (dap), the Bali Karet and Bima Brebes had the highest rate of armyworm attack on shallot plants, in contrast to other cultivars. The severity of the attack is directly proportional to the age of the plant. The peak of the biggest attack occurred 56 days after planting, when the Bali Karet harvest reached 46.2%. Most of the plants, especially the Bali Karet cultivar, lost their leaves as a result of the severe attack. Despite their modest size, almost all plants are capable of producing bulbs. *S. exigua* attack eats the inner leaf surface and leaves the outer epidermis (Figure 4).

**Figure 4.** Symptoms damage by *Spodoptera exigua* larvae on shallots cultivars in this study

**Table 2.** The development of *Spodoptera exigua* population according to phenology of several shallot cultivars

| Cultivars | Day After Planting (DAP) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 | 70 | 78 | 82 |
| | Number of larvae | | | | | | | | | | |
| Bima Brebes | 0 | 0.0 a | 0.0 a | 0.8 a | 0.8 a | 0.0 a | 0.6 a | 1.6 a | 1.2 a | 1.2 a | 1.0 a |
| Bauji | 0 | 0.0 a | 0.0 a | 0.0 a | 0.0 a | 0.0 a | 0.8 a | 0.8 a | 0.4 a | 0.4 a | 0.6 a |
| Tajuk | 0 | 0.0 a | 0.0 a | 0.4 a | 0.4 a | 0.0 a | 0.4 a | 0.6 a | 0.8 a | 0.6 a | 0.2 a |
| Probo | 0 | 0.0 a | 0.0 a | 0.2 a | 0.4 a | 0.0 a | 0.6 a | 0.6 a | 0.8 a | 1.0 a | 0.8 a |
| Bali Karet | 0 | 0.0 a | 0.2 a | 0.0 a | 0.6 a | 0.0 a | 1.0 a | 1.2 a | 1.8 a | 1.6 a | 1.4 a |

Note: Different letters in the same column indicate significant differences between cultivars ($p < 0.05$).

**Table 3.** Attacked of *Spodoptera exigua* development according to several phenology of shallot cultivars

| Cultivars | Day After Planting (DAP) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 | 70 | 77 | 82 |
| | % of plants infected | | | | | | | | | | |
| Bima Brebes | 0 | 1.2 b | 3.2 a | 6.4 a | 14.0 a | 25.4 a | 31.8 b | 38.8 a | 37.0 ab | 31.8 b | 33.4 b |
| Bauji | 0 | 0.2 b | 4.2 a | 5.8 a | 6.8 a | 21.4 ab | 28.2 bc | 23.4 b | 22.4 c | 23.0 c | 24.6 c |
| Tajuk | 0 | 0.8 b | 3.8 a | 10.6 a | 10.6 a | 15.6 ab | 27.0 bc | 26.4 ab | 21.2 c | 20.0 c | 19.4 c |
| Probo | 0 | 0.6 b | 2.2 a | 7.2 a | 7.2 a | 7.2 b | 18.2 c | 30.8 ab | 30.8 bc | 33.2 b | 33.6 b |
| Bali Karet | 0 | 2.8 a | 5.4 a | 14.2 a | 14.2 a | 25.4 a | 46.2 a | 41.0 a | 43.2 a | 43.0 a | 42.4 a |

Note: Different letters in the same column indicate significant differences between cultivars ($p < 0.05$).

Our data demonstrate that the plant age of diverse shallot cultivars has a considerable impact on the attack rate of *S. exigua*. The Bali Karet cultivar was the most attacked until the plant was 82 (dap). *Spodoptera exigua*, interestingly, may infect plants from the early development stage (1-10 dap) through the tuber maturity stage (51-65 dap). The immature armyworms (instar-1) then promptly puncture the tips of the leaves and enter into the leeks, consuming the inner surface of the leaves. The leeks will then become pale, with white translucent spots, and the leaves will droop (Putrasamedja et al. 2016).

Several cultivars of shallots were also used to measure the attack rate of *S. exigua* (Zheng et al. 2000). Previous research suggested that the poor survival of *S. exigua* larvae reflected the caterpillar's limited assault on shallot plants in the field (Mehrkhou et al. 2012). As previously stated, the nutritional value of host plants has a significant impact on the growth, development, and reproduction of herbivorous insects (Clissold and Simpson 2015), so the availability of suitable host plants plays an important role in the spread of insect outbreaks (Skendžić et al. 2021). However, a more extensive investigation on essential features of the nutritional content of the Bali Karet variety that is able to impact the attack and physiology of *S. exigua* in this study is still required. However, the quality and amount of food ingested during the first few instars and the rest to sustain insect metabolism, which varies depending on the host plant, might contribute to increased total insect attacks (Barros et al. 2010). As a result, population size and attack rate are critical markers of insect population dynamics on a certain host plant (Liu et al. 2004).

**Molecular characteristics of *Spodoptera exigua***

The results of PCR amplification based on the COI gene in both samples showed the same DNA banding pattern at ±670 bp (Figure 5). The results of the sequencing showed that the two isolate samples in this study were similar to *Spodoptera exigua* (100% similar).

The results of genetic distance analysis between Se0 and Se2 samples and nucleotide sequences of the COI gene of *S. exigua* from other countries obtained from GenBank, namely as ingroup *S. exigua* (China, Japan, Thailand, India, Pakistan, Australia, North America, Mexico, Germany), and outgroups *S. mauritia* and *S. litura*. *Spodoptera exigua* genetic distance from Kintamani Se0 and Se2 was 0.000, indicating that there were no genetic differences based on the 100% identical COI gene (Table 4). Based on the findings of the two samples' alignment, which revealed no nucleotide differences, the genes held by the two samples based on the COI gene were 100% identical, as shown by the genetic distance of 0.000. (0%). The genetic distance implies that the stronger the genetic similarity, the lower the genetic distance between the species, or opposite.

Phylogenetic analysis showed *S. exigua* in Kintamani was in the same group as *S. exigua* from China, India, Pakistan, Thailand and Japan (Figure 6).

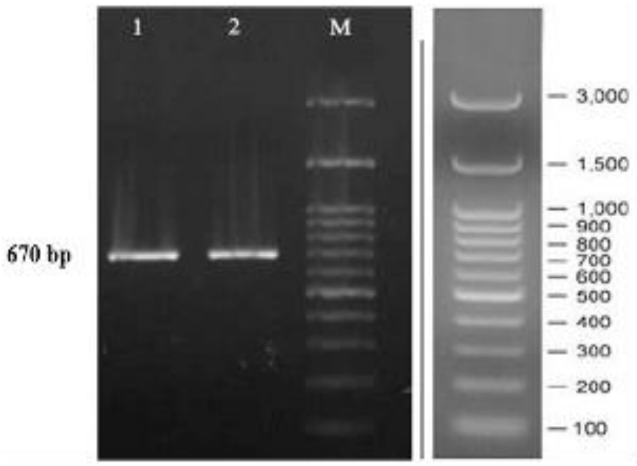


**Figure 5.** DNA band amplicon of the COI gene of *S. exigua* larvae. Note: 1: Sample 1 (Se0); 2: Sample 2 (Se2); M: Marker

**Table 4.** The genetic range of the *Spodoptera exigua* COI gene in this study

| GenBank acces number | Genetic distance | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| MK318332 | | | | | | | | | | | | |
| EU779856 | 0.000 | | | | | | | | | | | |
| KX281220 | 0.000 | 0.000 | | | | | | | | | | |
| Se0 | 0.004 | 0.004 | 0.004 | | | | | | | | | |
| Se2 | 0.004 | 0.004 | 0.004 | 0.000 | | | | | | | | |
| AB733674 | 0.004 | 0.004 | 0.004 | 0.000 | 0.000 | | | | | | | |
| FN908004 | 0.004 | 0.004 | 0.004 | 0.000 | 0.000 | 0.000 | | | | | | |
| KX861867 | 0.004 | 0.004 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | |
| JX316220 | 0.004 | 0.004 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | |
| JQ064572 | 0.004 | 0.004 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | |
| AB733409 | 0.122 | 0.122 | 0.122 | 0.122 | 0.122 | 0.122 | 0.122 | 0.122 | 0.122 | 0.122 | | |
| KF022223 | 0.090 | 0.090 | 0.090 | 0.090 | 0.090 | 0.090 | 0.090 | 0.090 | 0.090 | 0.090 | 0.088 | |

Note: Ingroup: MK318332: *S. exigua* Meksiko; EU779856: *S. exigua* Australia; KX281220: *S. exigua* Amerika Utrara; JQ064572: *S. exigua* India; FN908004: *S. exigua* Thailand; AB733674: *S. exigua* Jepang; KX861867: *S. exigua* Pakistan; Se0: *S. exigua* 1, Se2: *S. exigua* 2; JX316220: *S. exigua* China. Outgroup: AB733409: *S. maurita* and KF022223: *S. litura*

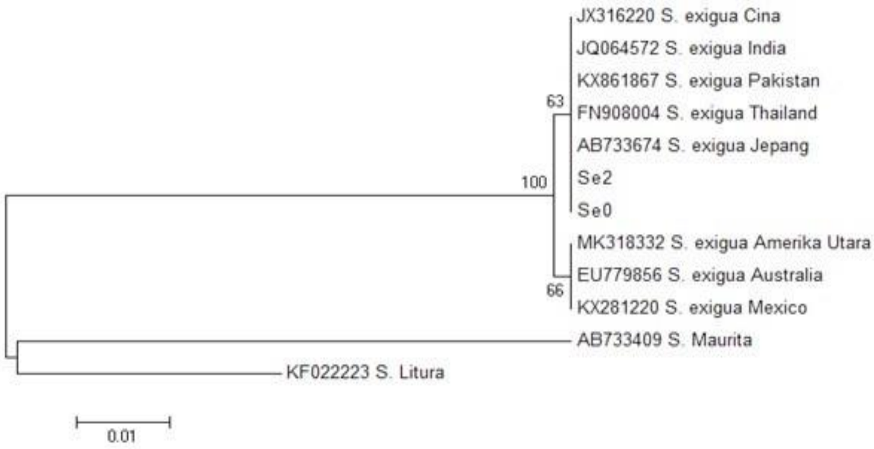


**Figure 6.** The phylogenetic tree was developed based on the COI gene using the Maximum Likelihood method (1000×; Tamura-Nei model). *Spodoptera exigua* isolates collected from this study were coded Se0 and Se2

We confirm that the presence of *S. exigua* in this study is the first report confirming its presence in the various shallots cultivars tested. This study also revealed that the two *S. exigua* isolates that attack shallots cultivars are closely related to several *S. exigua* species from several countries in Asia. The emergence of *S. exigua* in this study was probably caused by the export-import activities of shallot agricultural products to various destination countries. Reports show that the spread of invasive pests in China can occur through two main migration routes, namely the western route (Myanmar) and the eastern route (Indochina) (Li et al. 2020). In this study, it is assumed that the distribution of *S. exigua* in shallot plantations in Bali Province occurs along the routes of China, India, Pakistan, Thailand, and Japan because geographically, these five countries have close proximity to areas in Indonesia, including Bali Province. Given the vast territory of Indonesia, it is very possible that this pest originates from a domestic region/province, giving rise to speculation that still needs to be proven.

Overall, the invasion of *S. exigua* has been seen since 7 (dap), with the colonization phase beginning at 21 (dap), exactly when the larvae began to grow and develop in the field. The shallot variety also impacted the growth of the larval population of *S. exigua*, with the Probo cultivars being the most resistant and the Bali Karet and Bima Brebes kinds being the most vulnerable. Similarly, the kind of shallot cultivars impacted larval attack, with the Probo cultivars being the most resistant and the Bali Karet cultivars being the most vulnerable. As a result, it is proposed that using more resistant to *S. exigua*, such as the Probo cultivars, might be a viable solution to lessen the issue of attack and decline in farmers' crop yield caused by *S. exigua* in Indonesia, especially the Bali Province. This study also found and confirmed the presence of *S. exigua* invading shallot cultivars, and this is the first report with molecular characteristics of the appearance of the isolate in the province of Bali, Indonesia. Interestingly, the two isolates have close kinship with isolates reported from several countries in Asia, such as China, India, Pakistan, Thailand, and Japan. As a result, it is advised that more studies be conducted using biological agents such as natural enemies and the biological activity of plant extracts and comparing them to presently available commercial pesticides.

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