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Population Dynamics of White Grub, *Holotrichia seticollis* (Coleoptera: Scarabaeidae) in NW Himalayan Regions of India

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Abstract: Population dynamics analyses of *Holotrichia seticollis* Moser, 1912 (Coleoptera: Scarabaeidae) through the light trap, pheromone trap and in-situ sampling were conducted in Uttarakhand, Himalayas for three consecutive years (2019-2021). The three chemical synthetic attractants tested for their efficacy in attracting and trapping the adult males of *H. seticollis* showed that the most potent species-specific synthetic attractant was methoxybenzene that trapped up to 14.58 beetles/day, followed by diethyl benzene (1.29) and 1, 4- diethyl benzene (0.53). Second fortnight of June was peak period for the emergence of *H. seticollis*. The maximum trap catches were between 21 to 26 standard meteorological weeks (SMWs), while a sharp decline in population after 26 SMW in both the traps as well as in-situ collection. However, the light trap catches were extremely low in comparison to pheromone traps, thus indicating that, although *H. seticollis* is a nocturnal insect, but it was not positively phototactic. Therefore, pheromone traps can be successfully used for monitoring pest abundance of *H. seticollis* at field level and also assist in drawing accurate risk maps for designing and implementing sustainable and eco-friendly integrated pest management programs in the Indian Himalayas.

Keywords: *Holotrichia seticollis*, Para-pheromone, White grub management

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Order Coleoptera represents the largest group of insects with over 4,00,000 species (Beutel and Haas 2000). Amongst the different families of Coleoptera, Scarabaeidae is the second largest family with more than 30,000 species of cosmopolitan distribution all over the world (Jameson and Ratcliffe 2001). The majorities of the beetles belonging to the family Scarabaeidae are pleurostict (phytophagous) and commonly known as scarabs, whereas, their larvae are known as white grubs and are rhizophagous. Uttarakhand Himalayas, India constitutes the vast diversity of Scarabaeid beetles with more than 100 species reported to infect agriculture fields, horticulture crops and forest trees in the state (Mittal 2005, Chatterjee 2010 and Chandra et al 2012). Among these 100 species, *Anomala dimidiata* (Hope, 1831), *Holotrichia longipennis* (Blanchard, 1851) and *Holotrichia seticollis* Moser, 1912 are the three dominant species causing more than 50% of crop damages during the Kharif season every year (Selvakumar et al 2011, Subbanna et al 2020). *H. seticollis* is one of the predominant and most destructive scarab species causing considerable economic losses in agro-ecosystems of the North-Western Indian Himalayas (Malik et al 2019). Both, the grubs and adults are pestiferous and mainly attack crop plants such as potato, ginger, upland rice and sugarcane, in different regions of the country (Abdullah 2012, Chandel et al 2012, Padala et al 2017). The demand for efficient risk assessment techniques and accurate methods for monitoring the pest population dynamics of these notorious white grubs is increasing over the years, in order to implement timely and efficient pest management programs to avoid environmental risks. However, due to the cryptic habitat of the white grubs, it becomes difficult to evaluate their population dynamics and pest control turns to be a very difficult affair, thus, leading to unsatisfying results and leaching of inputs incurred. Beetle population monitoring could be useful for understanding the potential risk of white grub damage in a particular area. Given the economic importance of *H. seticollis* in the Indian Himalayas, a simple and effective strategy for monitoring the population density and dynamics of *H. seticollis* is necessary, as it would yield us a correct and perfect timing for planning an effective and environmentally sustainable wide-area IPM program.

For many decades, light traps were primarily used to monitor the abundance and diversity of positively phototactic and nocturnally active insect species in a particular habitat (Hong et al 2021). Off late, the mechanism of chemical communication among the individuals of an insect species is exploited, by developing the synthetic analogs of the so-called "pheromones" released by insects which are species-specific, less laborious and more economical with no extra external inputs like power as in case of light traps (Mullen and Dowdy 2001). These synthetic sex pheromones used in the traps mainly include sex or aggregation pheromones, that play an important role in the lives of many insects and are exploited as monitoring tools for estimating the pest population dynamics and ultimately in their management (Baker and Heath 2005). Although, recently the database on insect pheromones and other attractants has been developed by several authors but the studies to exploit them in insect attraction and management have mostly been confined to laboratory levels (El-Sayed 2008). Additionally, the database on pheromone/attractants specifically-related to notorious white grub species belonging to the same genera *i.e., Holotrichia consanguinea* (Leal et al 1996) and *Holotrichia reynaudi* (Ward et al 2002) are available. But, studies specifically related to detecting the *H. seticollis* population at an early stage of infestation in order to maintain low pest densities and avoid serious pest outbreaks are not available. In view of this, the present study on quantitative monitoring of *H. seticollis* populations using light traps and pheromone traps as lures has been carried out at field levels in the NW Indian Himalayan region. Further studies to identify the response of adult males of *H. seticollis* to different synthetic chemical attractants and light traps were conducted and the most efficient trap for monitoring the population dynamics and mass trapping of insect pests was identified. In addition to these studies, daily in-situ field surveys were conducted in the study area to confirm the accuracy of the catch of both light and pheromone traps installed in the field.

MATERIAL AND METHODS

Study site: The present investigation was carried out for three consecutive years (2019-2021) from the 20th to 33rd standard meteorological weeks (SMWs) in Experimental Farm, ICAR- Vivekananda Parvatiya Krishi Anushandhan Sansthan (29.64º N, 79.63º E and 1284 m above mean sea level), Hawalbagh, Almora, Uttarakhand, India. The study location comes under the Alpine and Humid subtropical climatic zone of the NW Indian Himalayas.

Mating cycle of adult *H. seticollis*: In order to understand the important timelines in the mating cycle of adult *H. seticollis,* the time of female emergence, settlement, male calling, mating, uncoupling and egg-laying were recorded from more than 100 pairs of *H. seticollis.* The current data was used for extraction of the natural pheromone from adult females through a sampling apparatus for in-situ volatile collection (Patent number: IN 373714) (Fig. 1).

Screening of synthetic attractants: The natural pheromone extracted through a handheld headspace sampling apparatus for the in-situ volatile collection was identified as 1,2 1,3 and 1,4 diethyl benzene through Gas chromatography Mass spectrometry. The three synthetic analogs, diethyl benzene, 1,4- diethyl benzene and methoxybenzene of 1,2 1,3, and 1,4 diethyl benzene were tested as lures in preliminary field trials in the year 2019. All these chemical attractants are commercially available and obtained from Himedia Laboratories Pvt. Ltd., India. The most potent species-specific synthetic attractant among the three was used for further comparative study with the light trap. In addition to this, the effect of these synthetic attractants on the non-target and beneficial insect species was also evaluated.

Light trap vs. pheromone trap: In the present study, two types of traps (light trap and pheromone trap) were used for monitoring the population dynamics of adult beetles of *H. seticollis* in the study area. The light trap (VL white grub beetle trap-1; Indian patent number: IN 290170) is specifically designed to attract and trap the scarab beetles (Fig. 2a). The trap is designed based on a simple mechanism of attracting positively phototactic and nocturnally active insects. The hitting fins fixed at an angle of 120º increase the efficiency of the trap, wherein, the scarab beetles hovering around the light source hit the fins and fall into a collection vessel fixed at the bottom through the Y-shaped vessel. The collection vessel is half-filled with water in order to avoid the escape of the trapped beetles. The pheromone trap was identical to the VL white grub beetle trap-1, except, the light source was replaced with the synthetic pheromone (Fig. 2b). The synthetic pheromone (500 µl) is filled into a 1.5 ml plastic vial with needle holes at the cap and fixed onto the trap. The scarab beetles get attracted to the pheromone and hover around this synthetic attractant. During this process, get hit by the hitting fins and fall into the collection vessel. The synthetic pheromone was replaced every 7 days.

Both the traps were installed in the first week of May, to record the information on the date of the first emergence, time of emergence, time of maximum activity, population density and population abundance of adults of *H. seticollis*. Moreover, the pheromone traps and light traps were installed at a height of 1.0 m from the ground level and at a distance of 300 m in order to minimize inter-trap interference.

In-situ sampling: In-situ sampling of adults, *H. seticollis* was also carried out simultaneously, by scouting the fields from 19:00 to 21:00 hrs on daily basis in the study area during 2019-21, in order to confirm the accuracy and reliability of light and pheromone trap catches. Apart from the monitoring study, the mating pairs per unit area (10 m^2) were also observed and the decline in the number of mating pairs over a period of three years was also assessed. In addition to this, the number of unmated females was also observed and an increase in the number of unmated females over a period of three years was also assessed to observe the effect of pheromone traps on mating disruption.

Data collection: The emergence of adult beetles from soil commences in the evening hours immediately after receipt of the first pre-monsoon rains. They emerge in large numbers for feeding and breeding. Considering the activity of beetles, the traps were operated between 19:00-6:00 hrs daily and the in-situ sampling was done from 19:00-21:00 hrs for three consecutive years (2019-21). During this time period, the beetles were actively mating and feeding on their host plants. The beetles collected through both methods were sorted out and the numbers of males of *H. seticollis* were recorded separately and the total number of adults of *H. seticollis* captured by all means were counted and noted on daily basis.

Data analysis: The SE (m) values were calculated through SPSS software for WINDOWS version 16.0 (SPSS Inc., Chicago).

RESULTS AND DISCUSSION

Reproductive behavior and mating cycle of *H. seticollis*: The emergence of *H. seticollis* starts immediately after the first pre-monsoon showers, during the second fortnight of May and continues till the first fortnight of August. The adults emerge from the soil for feeding and mating at 19:15-19:30 hrs and settle on nearby host plants (leaves of *Rosa indica,* bark of *Cedrus deodara*, *Dalbergia sissoo* and *Thuja occidentalis*) (Fig. 3a, b). The adult female settles on the host tree, protrudes its pheromone gland and releases male attracting sex pheromone, which attracts a large number of males. The sexually active time of *H. seticollis* was observed to be between 19:10-19:40 hrs and mating occurs for 10 minutes. Immediately after mating, both the males and females uncouple themselves and the females return to the soil for egg-laying, while, the males were observed to move to their host plants for feeding.

Screening of synthetic pheromones/ parapheromones: In order to assess the effectiveness of three chemical attractants (diethyl benzene, 1,4- diethyl benzene and methoxybenzene) in attracting and trapping the adult males of *H. seticollis,* three traps lured with three different chemical attractants were installed in the field at a minimum distance of 300 m. The most potent species-specific synthetic attractant was methoxybenzene (anisole, $CH_3OC_6H_5$) that trapped up to 14.58 beetles per day (Fig. 3c), followed by diethyl benzene (1.29 beetles) and 1,4- diethyl benzene (0.53 beetles), respectively (Fig. 4). The attraction for anisole was stronger than the actual pheromone released from female of *H. seticollis* and this synthetic parapheromone masked the activity of natural pheromone, thus, disrupting the mating process of *H. seticollis*. Moreover, none of the traps lured with synthetic attractants trapped any non-target or beneficial insects. Methoxybenzene was used for further comparative study with the light trap and in-situ samplings.

Light trap v/s pheromone trap v/s in-situ sampling: Species sampling is a basis for documenting the spatial distribution of species in an ecosystem (Zhang 2011). A simple and effective method is very important to estimate the abundance and population dynamics of an insect species in a particular habitat (Southwood and Henderson 2000). A large number of trap designs are commercially available and have

Fig. 1. Natural pheromone extracted through a handheld headspace sampling apparatus for in-situ volatile collection

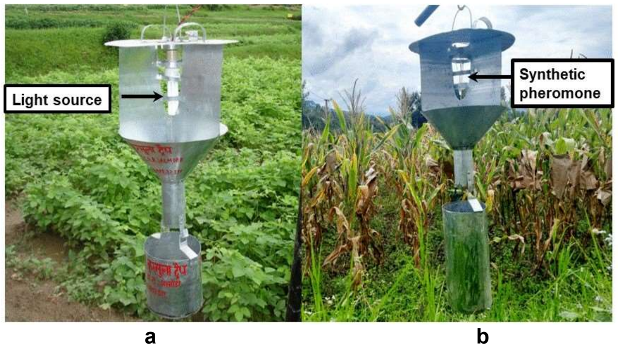

Fig. 2. Traps used for monitoring the population of *H. seticollis*; (a) VL white grub beetle trap-1; (IN 290170) and (b) Pheromone trap

Fig. 3. *Holotrichia seticollis*; (a) Matting pairs settled on a tree trunk, (b) Feeding on a host tree and (c) Trapped in a pheromone trap with a synthetic attractant (methoxybenzene) as a lure

been tested for the detection, monitoring and control of various insect pests (Ávalos and Soto 2015, Fite et al 2020). Both light and pheromone traps were examined for their efficiency in trapping the adult beetles of *H. seticollis.* The second fortnight of June was the peak period for the emergence of *H. seticollis* (Fig. 5). Sreedevi et al (2014) also reported the peak emergence of scarab beetles during the second fortnight of June. The highest pheromone trap catches were between 21st to 26th SMWs, while, a sharp decline in population was observed after 26th SMW in both the traps as well as in-situ collection. The light traps showed the least activity in trapping the adults of *H. seticollis*, thus indicating that, although, *H. seticollis* is a nocturnal insect but it was not strongly phototactic. Although, previous studies conducted by Dhaliwal and Arora (2010), Banjar et al (2020) and Menis and Rodrigues (2021) showed that the light trap is the best sampling method for monitoring the population density of nocturnal white grub beetles which are positively heliotactic in nature. But, *H. seticollis* was not strongly phototactic and thus the use of the light trap to assess the population dynamics of *H. seticollis* in the Indian Himalayas is not the right strategy.

Pheromone traps were highly efficient & extremely species-specific and the trap catches were observed at least a week or two earlier than the light trap when the population of *H. seticollis* was supposed to be very low. The pheromone trap catches recorded activity from 20th to 32nd SMWs during the entire activity period of the beetles in all three years, whereas, in-situ collection studies carried out to confirm the activity of both traps showed that, the emergence of adult beetles was observed from 21st to 30th SMWs in 2019 and 20th to 30th SMWs in 2020 and 2021, respectively. Witzgall et al (2010) and Ahmad and Kamarudin (2011) also stated that pheromone traps are efficient even at low pest population densities with no adverse effect on non-target species and the long-term use of synthetic pheromones can lead to a reduction in pest populations. This study clearly indicates that pheromone traps are more efficient than light traps in detecting the presence of scarab beetle, *H. seticollis*. Both the traps as well as in-situ collection data indicated that the emergence of *H. seticollis* started during the second fortnight of May and the pest abundance increased till mid-June and the population density reduced from the last week of June (Fig. 5).

The number of mating pairs settled on a tree trunk of five *Cedrus deodara* trees in a $10\ m^2$ area in one day in three different localities were counted for three consecutive years and was observed that the number of mating pairs declined over the years (from 28.33 in 2019 to 24.33 in 2021) (Fig. 6). Moreover, the studies conducted at USDA APHIS (2011) reported that trapping of a large number of male adults through synthetic sex pheromones can result in an imbalance in the pest sex ratio and this may affect the mating pattern of the pests. Concurrently, our studies showed a continuous reduction in the mating pairs of *H. seticollis* over a period of three years, when methoxybenzene-lured pheromone traps were continuously used for trapping the adult males. Although, the decline was not drastic, but continuous use of pheromone traps may lead to population decline over the years.

Increase in number of unmated females: Number of unmated females increased over the years per unit area

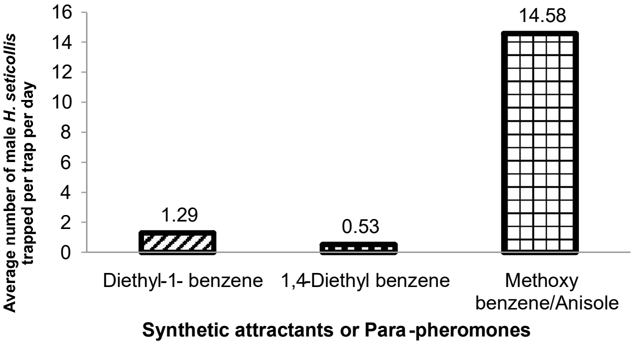

Fig. 4. Field efficacy of synthetic attractants used for trapping males of *H. seticollis*

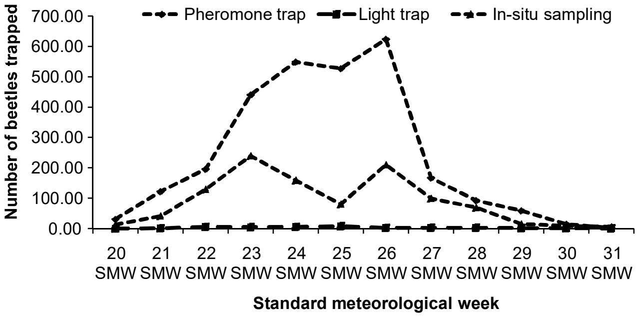

Fig. 5. Mean weekly trap catches of adult males of *H. seticollis* over a period of three years

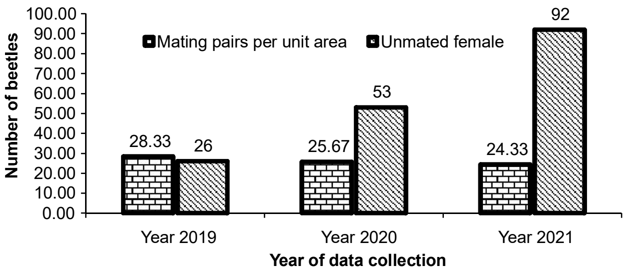

Fig. 6. Number of mating pairs and unmated females of *H. seticollis* collected during in-situ sampling during three years (2019-2021)

(Fig. 6) from 26 in 2019 to 92 in 2021. The continuous use of pheromone traps can lead to a significant increase in the number of unmated females and a decline in the number of mated females per unit area, which in turn reduces egg-laying and thus, lead to population reduction over the years. Furthermore, Kamarudin et al (2010), Muniyappa et al (2018) and Luo et al (2020) reported that mass trapping through pheromone traps, not only controlled the male population but, also efficiently reduced the larval population in subsequent generations, thus, resulting in a drastic decline in crop damage and yielding better quality products. Ward et al (2002) extracted the female pheromone from the abdominal glands of *H. reynaudi* and also tested three parapheromones; anisole, indole and phenol (singly and as binary mixtures) and recorded that no beetles were trapped in indole or phenol-baited traps and thus, concluded that, anisole is the major component of the female sex pheromone and plays a major role in attracting males of the same species. So, for all three species anisole is a sex pheromone. Moreover, the adult scarab beetle population above the ground can be positively correlated with the population of white grubs in the soil; this correlation could be utilized for pest risk assessment in a particular area. Therefore, the adult population levels assessed through pheromone traps can be utilized as a single risk assessment factor for crop plant damage by pest species (Furlan et al 2020).

CONCLUSIONS

The results of the present study provide one of the very first demonstrations of the use of pheromone traps for accurately monitoring the population dynamics of the target insect and obtaining a reliable and consistent estimate of the pest risk by *H. seticollis*. The pheromone trap with anisole as a synthetic attractant was identified as the best monitoring and trapping method against *H. seticollis*. So, this eco-friendly and cost-effective technology can easily be adapted by the farmers to monitor the pest population dynamics of *H. seticollis* on a wide area basis and take up early, timely and economic threshold-based pest management practices in the Indian Himalayas. However, few pheromones are not target-specific and attract all the insects of the same genera or sometimes beneficial insects, additionally; natural or synthetic pheromones are not available commercially for a large number of insects, which needs further research.

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