COMPARISON OF COLOR AND ATTRACTANT TRAPS EFFECT USED FOR SAMPLING APPLE BLOSSOM BEETLE (*TROPINOTA HIRTA* (PODA, 1761) (COLEOPTERA, SCARABAEIDAE, CETONIINAE))

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Abstract. This study was carried out to determine the most appropriate trap design for monitoring *Tropinota hirta* (Poda, 1761) (Coleoptera: Cetoniidae) among the commonly used traps in the world during the 2018. For this purpose, cherry gardens with three different loc. Atabey district, İslamköy and Pembeli villages of Isparta province were selected and the adult catch rates of the known binary attractant mixture (cinnamyl alcohol (3-phenyl-2-propen-1-ol) and transanethole [(1-methoxy-4-(1-propenyl) benzene)]) and white, light and dark blue color traps were compared with each other during the blooming period. As a result of the present study, the amount of adult catch of light blue color traps in the cherry blooming period was found to be significantly higher than the attractant and dark blue color traps which are regularly used in the world hence the use of this mentioned trap was found as the most effective biotechnical method for the controlling of *T. hirta* among the frequently used traps.
Keywords: *apple blossom beetle, attractant traps, cherry, color traps, Isparta*

Introduction

Apple blossom beetle has been expressed in the INPN which is the reference information system for data related to Nature (INPN) (https://inpn.mnhn.fr/accueil/a-propos-inpn) as *Tropinota hirta* (Poda, 1761) (Coleoptera: Cetoniidae) however the species every so often expressed as *Epicometris (Tropinota) hirta* (Poda, 1761) (57 scientific articles), *Tropinota* (*Epicometris*) *hirta* (33 scientific articles), *E. hirta* (11) and or *T. hirta* (12) and sometimes even expressed as under the family of Scarabaeidae instead of Cetoniidae.

Adults of *T. hirta* is polifagous and causing serious damage through feeding on pistils and stamens of the flowers of numerous plants such as apple, barley, blackberry, blackcurrant, broomrape, canola, cherry, lupine, narcissus, pear quince, plum, raspberry, rye, tulip, wheat, and other plants of agricultural importance even ornamental plants (Milenkovic and Stanisavljevic, 2003; Çetin et al., 2006; Ertop and Özpınar, 2011; Perez and Traveset, 2011). *T. hirta* may damage 70% of the blossoms on some plants of agricultural importance (Kutinkova and Andreev, 2004; Ražov et al., 2009). Control of *T. hirta* is difficult since most of the pesticide cannot applied during blooming period without affecting beneficial organisms that provide pollination, specially honey bees (Vuts et al., 2011a, b). Besides, long-acting pesticides also have an adverse effect on exports. Therefore, the application of biotechnical methods to control of the mentioned

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pest will be extremely beneficial both in terms of environment and human-animal health. Recent studies show that the most recommended biotechnical methods are color traps and color with attractant against *T. hirta* which is widespread in parts of Eurasia, from the Mediterranean to the Middle East and Central Asia. Baits have been used as biotechnical method to control and monitoring of *T. hirta* and it has been mostly prepared with *cinnamyl alcohol* (3-*phenyl*-2-*propen*-1-*ol*) and *transanethole* [(1-*methoxy*-4-(1-*propenyl*) *benzene*)] which compounds are common flower scent constituents by a 1:1 mixture (Knudsen et al., 1993).

Lot of valuable research have been conducted on controlling of *T. hirta* however it has been found that the results of scientific studies on the best biotechnical methods to be used for *T. hirta* have not corresponded with each other. Therefore the present study was conducted in order to find out the most suitable biotechnical methods for *T. hirta*.

Materials and methods

The study was carried out in cherry blooming period between April 30 and May 14 to compare efficiency of attractant (cinnamyl alcohol and transanethole) and color traps (dark blue, light blue and white) for sampling adults of apple blossom beetle adults during 2018.

Localities

Three different locations; (Atabey district (a: loc.1), (37°56'48.58"N/30°39'5.16"E) Pembeli (p: loc.2) (37°56'20.07"N/30°41'12.60"E) and İslamköy (i: loc.3) (37°54'51.77"N/30°39'14.76"E) villages in Isparta province, Turkey) were selected for the study. While color traps were placed in each locations with 10 pieces (3 colors × 10 pieces × 4 locations = 120 units), attractant traps were set up in Atabey district with 10 pieces.

Traps

Ten attractive traps (A) were placed on the cherry branches which are approximately 2 m high from the ground for the purpose of sampling of the adults of *T. hirta* in the cherry orchard in Atabey district, Isparta province, Turkey. Three different colors (hex code are inside brackets), light blue (LB) (#87CEFA), dark blue (DB) (#1E90FF), and white (W) (#FFFAF0), and 10 pieces for each one, were set up for three cherry orchards in Atabey, Pembeli and İslamköy (Isparta province). The ratios of *T. hirta* adults sampled from attractants and color traps were compared during the blooming period.

Baits were prepared with *cinnamyl alcohol* (3-*phenyl*-2-*propen*-1-*ol*) and *transanethole* [(1-*methoxy*-4-(1-*propenyl*) *benzene*)] by a 1:1 mixture according to the suggestions of Tóth et al. (2004) and Schmera et al. (2004) suggestions to catch *T. hirta*. A total of 10 units of cottons prepared in equal length were impregnated with attractive (1:1 mixture-20 µl) and placed inside the perforated (20 pieces with a diameter of 0.3 mm) falcon tubes (50 ml). Falcon tubes (3 × 13 cm length) with lures fixed with upside part of the light blue funnels (22 cm diameters and 25 cm length). Five liters (approx. 1.32 gallon) of plastic water containers which placed at the bottom of the funnels were used as traps to collect adults of apple blossom beetles. Totally 10 attractant traps prepared as mentioned above were hung tree branches nearly 2 meters

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above from the ground where the traps were a location representing the cherry garden. These attractive traps were placed only in Atabey district.

Color traps were designed as 25 cm in diameter and 10 cm in depth plastic basins. The order of colored traps were changed incidentally and set up 10 pieces for each locations. The distance between color traps were about one meter within a block and each block were set up 50 m distant from the next. 1/3 water was filled into the color traps to prevent adult of *T. hirta* from escaping. Blocks containing 3 traps with the above colors, were replicated 10 times, in the cherry orchard at each of the 3 locations. Each block the colors were distributed randomly hence the total number of traps was 90. Traps were set up on the soil surface in sunny places within the cherry orchards.

Both attractant and color traps were checked daily and the captured adults were counted and recorded.

Data analysis

All data from both adult numbers for each traps and groups of traps were analysed using ANOVA (one-way analysis of variance) and separated by Tukey's HSD (honestly significant difference) test at $P < 0.05$ to examine the differences between capture capability of attractant and color traps in SPSS.

Cluster analysis was performed using percent similarity and dendrogram based on the data was constructed with the help of MVSP software using the unweighted pair group method of arithmetic mean (UPGMA).

Results

It was found out that the number of individuals caught with color traps was found higher than individuals caught with attractant traps and differences was found out statistically significant. It was also compared the effects of the color traps with each other during the sampling adults of *T. hirta*. Light blue color traps were sampled more adults than the dark blue color traps.

Totally 776 individuals of *T. hirta* adults were sampled by attractant and color traps during the present study. The most of the proportion of *T. hirta* adults were caught by LB traps at Atabey, Pembeli, and Islamköy with 152, 136, and 126 individuals respectively. This means; nearly half of the specimens were sampled with LB traps at three loc. (Average: 138; individuals; proportion: 43.58%). The lowest of individuals were sampled by W traps at loc.1, loc.2, and loc.3 8, 12, and 11 respectively (Average: 10.33 individuals; proportion: 3.26%). Average of sampled adults of *T. hirta* were found similar with attractant and DB traps, with 87.0 and 81.3, respectively (Proportion: 27.47% and 25.68% respectively).

Capture rates of *T. hirta* adults with W set up at loc.1; loc.2, and loc.3 were found statistically different from the captures of other traps (*Table 1*).

It was determined that the least attractive trap for the adults of apple blossom beetle was W while the most attractive one was LB. Although no statistical differences were found between LBi with A, the adult capture averages of LBa and LBp were found statistically different from all other traps (*Table 1*). A was found not statistically significant with all DB traps at loc.1, loc.2, and loc.3 (*Table 1*).

Traps with the same color were grouped in terms of ease of understanding capture rates differences between color and attractant traps. Result of the capture means of all traps were given in *Table 2*.

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Table 1. *Comparison of capture rates of T. hirta adults sampled by attractant and color traps at loc.1., loc. 2, and loc. 3 in Isparta province*

Traps*	N	Mean ± Std. error	Std. deviation
Aa	10	8.7 ± 0.84 bc**	2.6687
LBa	10	15.2 ± 1.85 d	5.8652
DBa	10	8.6 ± 0.65 bc	2.0656
Wa	10	0.8 ± 0.25 a	0.7888
LBp	10	13.6 ± 1.34 d	4.2216
DBp	10	8.8 ± 0.74 bc	2.3476
Wp	10	1.2 ± 0.42 a	1.3166
LBi	10	12.6 ± 0.73 cd	2.3190
DBi	10	7.0 ± 0.67 b	2.1082
Wi	10	1.1 ± 0.31 a	0.9944

*A: Attractant; LB: Light Blue; DB: Dark Blue; W: White; a: loc. 1 (Atabey); p: loc. 2 (Pembeli); i: loc. 3 (Islamköy)
**According to a Tukey HSD test; means with the same letter in their superscript are not significantly different ($P < 0.05$)

Table 2. *Comparison of capture rates of T. hirta adults sampled by attractant and grouped color traps (values obtained from the same color were combined)*

Traps*	N	Mean ± Std. error	Std. deviation
Aa	10	8.7 ± 0.84 b	2.669
LB	30	13.8 ± 0.80 c	4.366
DB	30	8.13 ± 0.41 b	2.255
W	30	1.03 ± 0.19 a	1.033

*A: Attractant; LB: Light Blue; DB: Dark Blue; W: White; a: loc. 1 (Atabey); p: loc. 2 (Pembeli); i: loc. 3 (İslamköy)
**According to a Tukey HSD test; means with the same letter in their superscript are not significantly different ($P < 0.05$)

ANOVA test of data showed that no difference was found between the capture rates of A and DB traps ($P = 0.949$) however capture rates differences between them and the other traps (DB and W) were considered statistically significant (*Table 2*). The differences of catch proportions between DB-W and DB-LB traps were also found statistically significant ($P \leq 0.000$) (*Table 2*).

The dendrogram composed from the insect numbers sampled in all the set traps distinctly showed that all the same colors come together as a group while attractant trap was included with the DB color group (*Fig. 1*). Besides similarity values of A and DBa was found 90.17% as the most similar traps. It was seen that three main groups, W, LB, and DB with A were formed on the dendrogram. The similarity values of Wa and Wi traps captured by the least number of adults were seen as 63.15% (*Fig. 1*). Similarity values of Wp and the group formed by Wa and Wi was found 36.74% as the most dissimilar. The group formed by W traps at three localities showed 13.7% similarity with the group designed by all other traps, LB, DB, and A.

The following dendrogram is better at explaining dissimilarities and also shows statistically significant grades between groups. It was seen that A and DB traps were

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similar to each other by 84% while LB trap was found similar with this mentioned group by 74.9% (*Fig. 2*). W was equivalent to only with 19.2% of all other groups.

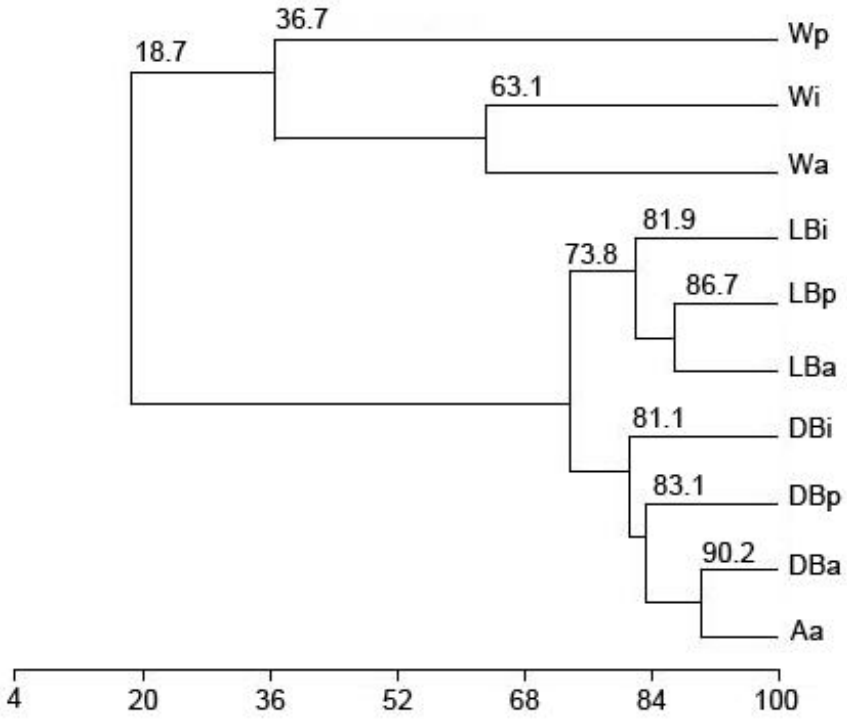

Figure 1. *Dendrogram of sampled T. hirta adults from the 9 colors and one attractant traps (A: Attractant; LB: Light Blue; DB: Dark Blue; W: White) in study locations (a: Atabey (loc. 1); p: Pembeli (loc. 2); i: İslamköy (loc. 3)). The cluster analysis was performed using Percent similarity*

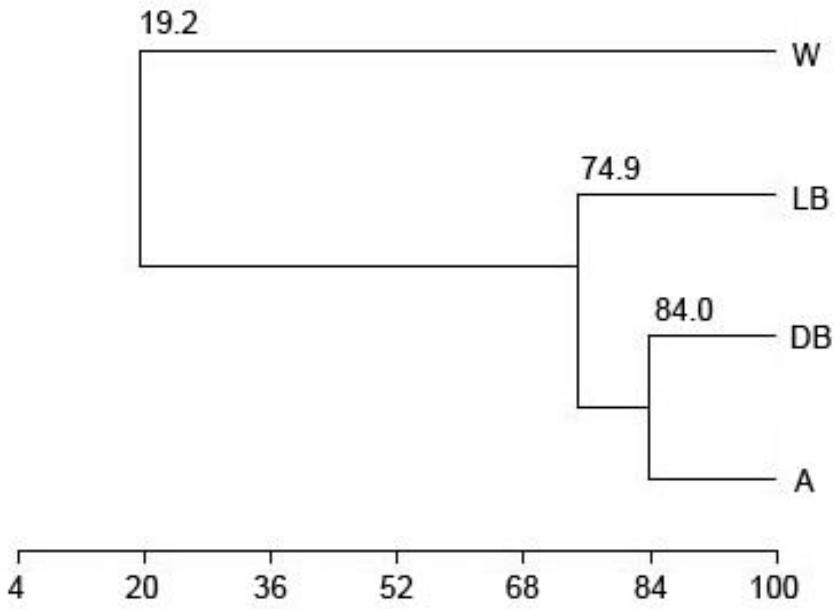

Figure 2. *Dendrogram of sampled T. hirta adults from the color traps (traps with the same color were grouped) and an attractant trap (A: Attractant; LB: Light Blue; DB: Dark Blue; W: White) in study area. The cluster analysis was performed using Percent similarity*

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Discussion

Many colors, yellow, fluorescent yellow, white, brown, transparent, chartreuse, coral, dodger blue, golden rod, hot pink, light sea green, light sky blue, medium orchid, etc have been evaluated to find out the most attractant colors for *T. hirta* in numerous scientific studies (Schmera et al., 2004; Kozár, 1972; Tóth et al., 2005, 2009; Aydın, 2011; Subchev et al., 2011; Vuts et al., 2012; Trotus et al., 2015). Some researchers declared that adults of *T. hirta* were strongly attracted to objects of light blue color (Kozár, 1972; Aydın, 2011) while some of them affirmed that the blue color has been the most attractive color for *T. hirta* (Tóth et al., 2005). Likewise some of the scientific study results showed that *T. hirta* had been attracted to yellow, light blue or white color in a field test, but they found no significant differences between colors (Schmera et al., 2004). On the contrary of the mentioned results, Aydin (2011) declared that the susceptibility of *T. hirta* to white and light blue colors was found statistically significant. We have tried three different colors of traps; white, light blue and dark blue during the blooming period of cherry orchard. Result of our study has showed that light blue color trap is the best attractant compared the other used traps for adults of apple blossom beetle and can be used for controlling of *T. hirta*

For the last decades researchers have tried different colors with different lures (Tóth et al., 2004, 2009, 1998, 2003; Schmera et al., 2004; Vuts et al., 2012; Trotus et al., 2015; Subchev et al., 2012) experiments were also carried out in different places at the same time (Vuts et al., 2010a). Cinnamyl alcohol, transanethol and cinnamyl acetate were found as compounds attracting *T. hirta* by Tóth et al. (2003). Thereupon Schmera et al. (2004) declared that combination of light blue color and a bait of 1:1 mixture of *cinnamyl alcohol* and *transanethol* used in dry funnel traps were very effective tool for catching of *T. hirta.* As for chemical attractive stimuli for *T. hirta*, a binary floral attractant consisting of (E)-cinnamyl alcohol and (E)-anethol has been optimised (Tóth et al., 2004, 2009, 1998, 2003). Finally a new coattractant, 4-methoxyphenethyl alcohol, developed for increasing the attractiveness of known binary attractant mixture, cinnamyl alcohol/trans-anethol (Vuts et al., 2010b). Vuts et al. (2010b) found that traps baited with the mixtures of known binary attractant with 4-methoxyphenethyl in 1:1:1 or 1:1:0.3 ratios sampled significantly more *T. hirta* than known binary attractant alone. However, Güvenç and Yaşar (2014) declared that there were no statistical differences between known binary attractant mixtures and known binary attractant with 4-methoxyphenethyl for catching of *T. hirta.* We have tried known binary attractant in our study. When we compare the effective of colors with mentioned attractant, almost similar number of individuals of *T. hirta* were caught with both attractant and dark blue color (87 individuals with attractant; 86, 88, and 70 with dark blue color in loc.1, loc.2, and loc.3, respectively) while the light blue color has been found the most effective tool for catching of adults of *T. hirta* (152, 136 and 126 individuals in loc.1, loc.2, and loc.3, respectively).

Some of the traps were used in the study of Yasar and Sagdas (2014) at apple orchards. Authors declared that the highest number of adults were obtained through the blue funnel trap in loc.1 however the most of the adults were caught by blue funnel + attractant traps when they compare with all four locations of the study. The results of our study coincide with study of Yasar and Sagdas (2014) however light blue traps were not include the mentioned previous study to compare efficiency of traps. Attractant and dark blue traps have been used detection and monitoring of apple

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blossom beetle on the other hand using light blue trap is highly recommended to control of *T. hirta* in cherry orchards according to our study results.

The study of Yasar and Uysal (2013) was conducted to evaluate the most effective traps for capturing *T. hirta* on plum and pear orchards. The most of the adults were sampled by blue funnel plus water traps with attractant (containing cinnamyl alcohol, trans-anethol, dipropylene glycol and 2 - propanol, 1, 1 - oxybis). They declared that use of an attractant with a blue funnel trap is an effective biotechnical method for controlling *T. hirta* when insecticides cannot be applied during the blooming period. Our study results showed that the attractant and dark blue color have not been found statistically different for catching adults of *T. hirta*. However the light blue traps caught the most individuals while the white color caught the least individuals when they compare to each other.

Gezer and Ozpinar (2015) were tested the different traps to determine the adult density of apple blossom beetle, in different orchards; peach, cherry, apricot and apple in Canakkale. They declared that the highest number of adults had been captured by the blue bowl plus water traps with attractant in all orchards. However there were no statistical differences between blue color bowl traps and blue color bowl traps with attractant containing trans-anethole + cinamyl alcohol, (1:1). It is an interesting result of the mentioned study that just blue bowl plus water trap was found the most effective than the all other traps with attractant. The authors explained this situation as the wind effect (Gezer and Ozpinar, 2015). Although we have got similar result with the mentioned study showed that blue color trap was the most effective traps, we believe that color is very effective tool for adult of *T. hirta* instead of wind condition instead of attractant.

The several scientific studies have been carried out on the subject and mentioned attractant mixtures have been used for controlling and/or seasonal monitoring of T. *hirta* in most of Central and Eastern Europe (Bulgaria, Hungary, Croatia and Italy) (Vuts et al., 2010a; Tóth et al., 2005, 2009; Subchev et al., 2011; Mircheva et al., 2004; Sivcev et al., 2006). We agree with these results that blue funnel with attractant trap can be suggested as an effective biotechnical method for controlling *T. hirta* however we believe that it is possible to control *T. hirta* without applying insecticide and population density of the mentioned species can be kept below the "economic damage threshold" by using the correct traps at the right time.

Some studies show that white color is the most attractive trap in terms of individual catching of *T. hirta* adults compared with light and dark blue color traps (Aydın, 2011). But some authors do not agree with this result and they declared that light or dark blue color has been found more attractive for the species than white color trap. On the other hand, the results of the studies carried out under different geographical conditions that do not coincide with each other as in the case of the study carried out in Central and Eastern Europe. The result of the study has been showed that CA-baited trap caught the most individual in Bulgaria, Hungary, and Croatia however scientists could not found the same result in Italy.

In fact, many studies have shown that the apple blossom beetle responds to different colors at different times. Scarcely, no studies reveal this result openly or expressed. It can be revealed that the color sensitivity of the beetle is in fact related to the plant phenology when all the results of the works are taken together instead of evaluating all the works separately.

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Result of our study showed that there is no statistically significant difference between the attractant and dark blue color whereas highest number of individuals caught with light blue color trap has been found statistically significant compared the other used traps. However, we are not sure that the same result can be obtained in studies to be done pre and post bloom period because we think that the color preference of the *T. hirta* changed depending on blooming periods. If behaviour pattern of apple blossom beetle is linked to plant phenology the color of traps should be changed considering the bloom period. Aydin (2011) has found same result with our study during blooming period of cherry however he has suggested using white color traps instead of traps with dark and light blue colors to catch more individuals of adult of *T. hirta* during pre and post blooming periods.

Results of many scientific studies and present study results showed that known binary attractant or known binary attractant with 4-methoxyphenethyl used with the light blue or dark blue colors cannot catch the similar number of individuals as the light blue traps which are set up at the ground surface. We think that the traps placed in the ground level can be more easily and earlier recognized than attractants hanged on branches of trees by *T. hirta*. Moreover it should be investigated that the rate of pest's flower damage on branches with an attractant hanging might be higher than the branches with not attractant hanging (Aydin and Yaşar, 2019). If pest is attracted to the trees with attractants damage rate can be increased. It may be more appropriate to set up the traps at a distance from the trees where adults of *T. hirta* can recognize easily rather than hang the traps on the branches.

Conclusion

There have been many scientific studies to understand which color or the attractant or even color and attractant use in conjunction is the best attraction method for sampling of *T. hirta*. When the studies conducted so far have been evaluated, we see that different sampling tools are suggested. Despite these confusing conclusions we wonder whether or not it is possible that the *T. hirta* color sensitivity changes at different times.

The second contradiction is that the necessity of using the attractors with color has not been explained in scientific studies. If the species is attracted to known binary attractant mixtures (or even known binary attractant with 4-methoxyphenethyl) why is the attractant has been recommended usage with color? It is understood from the related studies that only attractors do not affect the adults of apple blossom beetle. If the attractant used alone does not affect the insect and it is necessary to use it with color, so it can be said that the color is important. Some studies have emphasized that the use of color with known binary attractant and the use of only color (shades of blue) have not been found that has a statistically significant difference between their roles in attracting the insect. Our study results show that there is no difference between the number of individuals caught with the attractant and dark blue color trap. However, it is revealed that the number of individuals caught with light blue color is considerably higher than the others while the number of individuals caught with white color is very low. Both results have been found statistically significant.

The results of our study and the other related studies so far have been examined can be summarized as follows:

1. Plant phenology related shifts in color preferences of *T. hirta* adults.

1.1. Light blue color traps can be used during blooming periods of cherry orchards

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1.2. White color traps can be used during the pre and post blooming periods. The white color traps set up during pre-bloom can reduce the damage of *T. hirta* adults at present season while white color traps set up during post-bloom can reduce the population of the next year. Thus application order of this methods can be suggested both "as an effective biotechnical method for controlling *T. hirta*" and "population density of the pest can be kept below the "economic damage threshold".

1.3. Pesticide application against mentioned pest may not be needed with choosing right traps on time for monitoring of *T. hirta.* Thus non-target organisms such as honey bees, predator ladybirds, etc would not be negatively affected by applying insecticides.

2. Traps even including attractants should be installed in a visible place where the pest can easily recognize instead of hanging them on tree branches. Thus the rate of flower damage caused by the pest can be decreased and more numbers of adults can be caught

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