



# Assessing allelochemicals as species-specific attractants for the cherry bark tortrix, *Enarmonia formosana* (Lepidoptera: Tortricidae)

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## ARTICLE INFO

### Keywords:

Acetic acid  
Damage  
Kairomone  
Linalool oxide pyranoid  
*Malus domestica*  
Pear ester  
Integrated pest management  
2-phenylethanol  
Trapping

## ABSTRACT

Field trapping experiments were carried out in Norway to measure attraction of the cherry bark tortrix (CBT) *Enarmonia formosana* to volatile blends of candidate compounds including acetic acid (AA), linalool oxide pyranoid (LOXP), 2-phenylethanol (PET), pear ester (E,Z)2,4-ethyl decadienoate (PE) and (E)- $\beta$ -farnesene (BF). The binary blend of AA and LOXP caught the highest number of CBT adults. While addition of PET along with PE did not significantly change the attraction, a sex-dependent decrease of female captures was found when LOXP was replaced by PET/PE. Male attraction to AA/LOXP did not differ when PET/PE were added to the blend or when LOXP was substituted by the same two compounds. A similar attraction to blank traps was recorded for the ternary blend of LOXP/PET/PE, for the binary blend of PET/PE and for LOXP alone, supporting AA as a possible fundamental component of CBT kairomone. In addition, a lower number of bycatches of *Hedya nubiferana*, *Anthophila fabriciana*, *Synanthedon myopaeformis*, *Pammene* spp. and *Pandemis* spp. were scored in the AA/LOXP than in any blend including AA/PET/PE. BF was not behaviourally active on CBT in our field experiments. The high attraction of both sexes of CBT to the binary blend of AA/LOXP represents a first step towards the identification of a multicomponent kairomone for this pest. A continuous flight activity of both sexes of CBT was recorded from the end of May until the beginning of August, supporting the hypothesis that CBT is univoltine in Norway. Because larval infestation on tree trunks varies substantially with apple varieties, we encourage the collection of additional data to attempt a correlation between adult catch by AA/LOXP traps and the following larval population.

## 1. Introduction

Monitoring pest distribution, flight activity and damage to crops are recommended routines of integrated pest management (Barzman et al., 2015). Semiochemical baited traps are among the most widespread monitoring tools, because of their relatively easy to use properties. While sex-pheromones are active exclusively on males, allelochemicals volatiles are semiochemicals that can offer the advantage of attracting both sexes of phytophagous insects. This is particularly relevant in pests such as moths, where damages are inflicted by the larvae (Szendrei and Rodriguez-Saona, 2010). In these herbivores, plant and microbial volatiles play a major role in host-finding and oviposition choices of adult females. Host-derived volatiles can be used to monitor and predict the population size of egg-laying females into a crop. This is expected to refine current pest management practices and reduce pesticide use

(Knudsen and Tasin, 2015).

Several allelochemicals, either as single compound or as blends, have been identified as insect attractants (Bengtsson et al., 2006; Giacomuzzi et al., 2017; Tóth et al., 2009) and might be suitable to monitor female flight activity (Knight et al., 2019b; Knudsen and Tasin, 2015). Research on this topic focused predominantly on tortricids in orchards and vineyards, where new lures have been developed to target a number of species including *Archips xylosteana* (Linnaeus 1758) (Badra et al., 2019), *Choristoneura rosaceana* (Harris 1841) (El-Sayed et al., 2016), *Cydia pomonella* (Linnaeus 1758) (Basoalto et al., 2017), *Grapholita molesta* (Busck 1916) (Knight et al., 2014), *Epiphyas postvittana* (Walker 1863) (El-Sayed et al., 2016), *Lobesia botrana* (Denis & Schiffenmüller 1775) (Larsson Herrera et al., 2020a), *Pandemis* spp (Basoalto et al., 2017; Larsson Herrera et al., 2020b), and *Spilonota ocellana* (Denis & Schiffenmüller 1775) (Judd et al., 2017b) (Lepidoptera: Tortricidae). In

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<https://doi.org/10.1016/j.cropro.2020.105323>

Received 18 May 2020; Received in revised form 13 July 2020; Accepted 15 July 2020

Available online 22 July 2020

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some cases, blends of tested volatiles not only provided a high attraction to females, but also a higher potency on male than the corresponding sex-pheromone (Knight et al., 2019a). While some volatiles such as acetic acid (AA) and 2-phenyl ethanol (PET) are reported to be essential for the attraction of a number of tortricid leafroller species, other compounds such as pear ester (PE), linalool oxide pyranoid (LOXP) and benzyl cyanide seems to enhance specifically the response of some insect species (El-Sayed et al., 2016; Knight et al., 2019a; Larsson Herrera et al., 2020b).

CBT, *Enarmonia formosana* (Scopoli, 1763) is a native pest of trees of the Rosaceae family in Europe and part of Asia, while it has been established as an invasive pest in North America (Dang and Parker, 1990). Larvae of CBT feed under the tree bark, occupying the space between the cork and the cambium. Here, by causing loosening and cracking of bark, they expose the tree to secondary damage by the tree cankers and to an increased susceptibility to abiotic factors (Dickler et al., 1972). In Bulgaria, Toshova et al. (2017) considered CBT as an important pest of apple trees while Alford (2007) reported that it can eventually kill the tree. Sekse (1964) observed that CBT can cause serious damage in old pear trees in Western Norway. Recently, CBT damage on fruit trees was reported in Western Norway, where it was often associated with injury by fruit cankers (Børve J, personal communication). While a sex-pheromone is available to follow-up the male flight over the season (McNair et al., 1999), a specific lure capable of attracting both sexes of this pest has not yet identified and could provide key information to develop an integrated pest management program for this tortricid.

McNair et al. (2000) identified and tested plant volatiles from non-host plants (Trembling aspen, Grand fir, Norway spruce and Scots pine) and found that some volatiles had a repellent effect on CBT. A strong antennal response is reported for hexanol, benzyl alcohol, nonanal and decanal on both sexes. Adding these volatiles to traps baited with the sex-pheromone reduced the number of caught males. When these volatiles were added to a cherry branch, a reduction in the number of eggs laid by CBT was observed. Whereas knowledge on repellents is reported, studies aiming at the identification of possible CBT attractants are currently not available.

The objective of this study was to explore the behavioural response of CBT to candidate allelochemical volatile compounds as a first step towards the identification of a novel field attractant for this species. Based on recent studies on other tortricids, we selected generic (AA and PET) and specific (pear ester (E,Z)2,4-ethyl decadienoate (PE), LOXP and BF) volatiles and tested some of their combinations during 2017–2019 in some Norwegian apple districts.

## 2. Materials and methods

### 2.1. Volatiles

Synthetic glacial AA (700 mg; 99.8%; VWR Chemicals, Belgium), PET (700 mg; 99%; Acros Organics, China), LOXP (10 mg; 99.1% purity, 57.8% Z-isomer and 41.3% E-isomer, Nippon Terpene, Japan), and BF (10 mg; >90%, Sigma Aldrich) were loaded as single compound onto a dental cotton wick placed into a polyethylene vials with a 2 mm hole in the lid (Eppendorf; 2 ml) (see Larsson Herrera et al., 2020a for details). Dispensers were sealed into aluminium-plastic bags and kept at  $-18^{\circ}\text{C}$  until use. Grey halobutyl septa loaded with PE (3.9 mg) were obtained from Trècè Inc. (Salinas, CA, USA). A transparent delta trap with a sticky insert (Pherobank, BV, The Netherlands) was baited with volatiles and hung in the canopy of an apple tree at a height of 2 m. Distance between traps was 10 m.

### 2.2. Field trials

Field trials were conducted in several apple orchards in 2017 in Western Norway (Hardanger, N (traps) = 5, 100 m a.s.l.) and in 2018 in

Eastern Norway (Viken, N = 8, orchards = 3, 400 m a.s.l.). In 2019, field trapping was carried out in Viken and Hardanger (N = 9, orchards = 3). 'Aroma' was the main apple variety in all fields and all years. Five and seven different volatile combinations were compared in 2017 and 2018, respectively (see Tables 1 and 2). In 2019, only the best combination found in 2018, i.e. AA/LOXP, was used. The number of trapped adults (both target and non target moth species) was counted weekly from week 21 (May 24<sup>th</sup>) till 31 (August 2nd) in 2017, from week 22 (June 1st) to 32 (August 9th) in 2018, and every second week from week 22 (May 30th) till 31 (August 1st) in 2019. Whereas in 2017 only the total number of caught CBT adults was recorded, in 2018–2019 a distinction between sexes was also done. Unbaited traps were used as blank.

In addition to trap catches, a survey of damaged trees was recorded in 2019. Ten trees in each of ten rows (replicate) were visually inspected in each orchard. The lower part of the trunk was visually surveyed for fresh larval frass in late May-early June, following indications by Jenner et al. (2004), who showed that approximately 95% of all *E. formosana* larvae were collected from the trunk within 40 cm from the ground. The surveyed cultivars included Aroma, Discovery, Gravenstein and Summerred.

### 2.3. Statistics

Catch data were analysed and visualized with R (R Core Team, 2020). The number of caught insects was pooled across dates for each replication. The data was then fitted to a Poisson generalized linear model (glm) and tested for overdispersion using the package AER (Kleiber and Zeileis, 2008). When the data was significantly overdispersed, a negative binomial distribution, from package MASS, was used instead (Venables and Ripley, 2002). The package multcomp was used to discriminate among treatments (Hothorn et al., 2008). Treatments with no catches were omitted from the analysis.

## 3. Results

### 3.1. Attraction to tested volatiles

The results of catches in 2017 and 2018 are presented on [tbl1](#) Tables 1 and 2, respectively. No significant effect of treatment on the catches of *E. formosana* adults was found in 2017. Although a low population level did not allow for a statistical discrimination among treatments, the blend of AA/LOXP showed the highest captures ( $2.2 \pm 4.4$ ). The AA/BF combination caught  $1.2 \pm 1.1$  moths, while  $0.8 \pm 0.8$  were scored in the ternary blend with AA/PET/PE. A lower number of adults ( $0.4 \pm 0.9$ ) were attracted to the blend with a 4-component blend of AA/PET/PE/BF. No CBT were observed in blank traps. Bycatches of *Pammene* spp. were recorded in all traps, although the highest numbers were recorded in AA/PET/PE with or without BF. A few *Pandemis* spp. were scored in traps baited with AA/PET/PE with or without BF and to a lower extent, in traps baited with AA/LOXP or AA/BF.

The number of catches did not differ significantly between each other. Catches of *Hedya nubiferana* were observed in traps baited with combinations including PE (AA/PET/PE and AA/PET/PE/BF) and in a lower amount in traps baited with the AA/LOXP blend. There were no catches of *H. nubiferana*, *Pammene* spp. and *Pandemis* spp. in unbaited traps.

In 2018 the highest number of females and males CBT were caught in traps baited with AA/LOXP, which did not differ significantly from the lower catches in traps with the more complex blend of AA/LOXP/PET/PE. Only AA/LOXP caught a significantly higher number of females than AA/PET/PE, which did not differ from the abovementioned four-component blend. Treatments without AA caught a similar number of females and males, which did not differ from those in blank traps (Fig. 1). A similar result was measured for males, with AA/LOXP catching a higher but not significantly different number of individuals than AA/LOXP/PET/PE or AA/PET/PE. Captures by the other blends did

**Table 1**

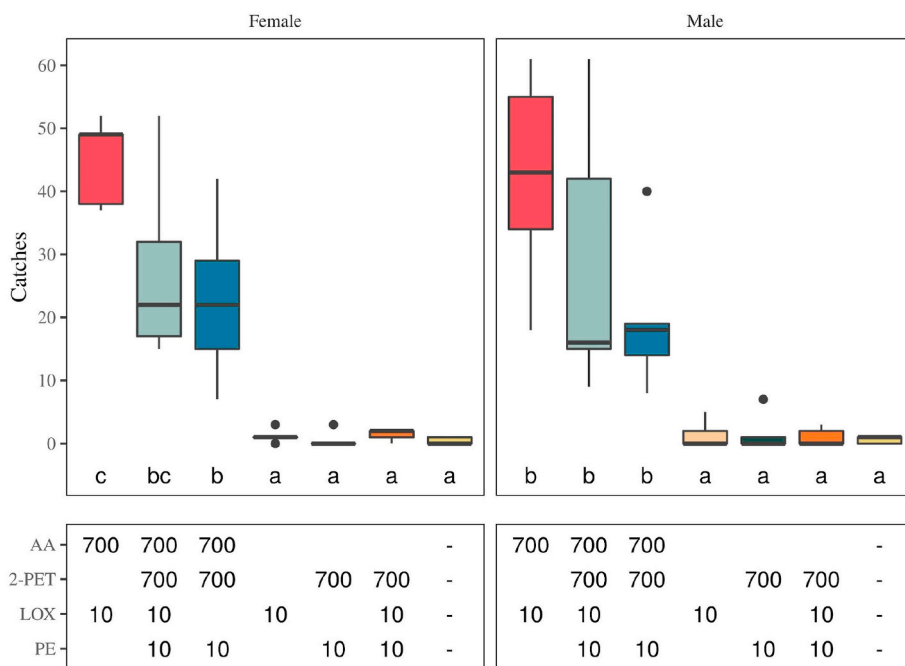
Catches of *E. formosana* in traps baited with volatiles in 2017. Compounds and their combination in treatments 1, 3 and 7 were also tested in 2018. Treatments 2017\_1 and 2017\_2 were only tested in 2017. The lower part of the table shows the total catch of each species in 2017 and information regarding the resulting glm models; Stat: statistical distribution, P-val: probability value for overdispersion with Poisson distribution, X<sup>2</sup>: chi-square value for factor treatment, P (X<sup>2</sup>): probability for the difference between treatments.

Compound						1	3	2017_1	2017_2	7
Acetic acid, mg						700	700	700	700	–
2-phenyl ethanol (PET), mg						–	700	–	700	–
Linalool oxide pyranoid (LOX), mg						10	–	–	–	–
Pear ester, mg						–	3.9	–	3.9	–
(E)-<beta>-farnesene, mg						–	–	10	10	–
Species	Sex	Stat	P-val	X <sup>2</sup>	P (X <sup>2</sup> )	1	3	2017_1	2017_2	7
<i>Enarmonia formosana</i>	Total	Poisson	0.35	7.7	0.053	11 a	4 a	6 a	2 a	–
<i>Hedya nubiferana</i>	Total	Poisson	0.089	10.6	0.005	5 a	13 ab	–	21 b	–
<i>Pammene</i> ssp.	Total	Poisson	0.066	15.4	0.001	7 a	25 b	7 a	14 ab	–
<i>Pandemis</i>	Total	Poisson	0.69	7.2	0.065	3 a	8 a	1 a	6 a	–

**Table 2**

Catches of *E. formosana* in traps baited with volatiles in 2018. The composition of the tested treatments is shown in the upper part of the table. The lower part of the table shows the total catch of each species and information regarding the resulting glm models; Stat: Poisson or negative binomial (NB) distribution, P-val: probability value for overdispersion with Poisson distribution, X<sup>2</sup>: chi-square value for factor treatment, P (X<sup>2</sup>): probability for the difference between treatments.

Compound						1	2	3	4	5	6	7
Acetic acid (AA), mg						700	700	700	–	–	–	–
2-phenyl ethanol (PET), mg						–	700	700	–	700	700	–
Linalool oxide pyranoid (LOX), mg						10	10	–	10	–	10	–
Pear ester (PE)						–	3.9	3.9	–	3.9	3.9	–
Species	Sex	Stat	P-val	X <sup>2</sup>	P (X <sup>2</sup> )	1	2	3	4	5	6	7
<i>Enarmonia formosana</i>	Female	NB	0.017	349.9	<0.001	225 c	138 bc	115 b	6 a	3 a	7 a	2 a
<i>Enarmonia formosana</i>	Male	NB	0.003	213.7	<0.001	211 b	143 b	99 b	7 a	8 a	5 a	3 a
<i>Enarmonia formosana</i>	Total	NB	0.007	368.2	<0.001	436 c	281 bc	214 b	13 a	11 a	12 a	5 a
<i>Anthophila fabriciana</i>	Total	NB	0.042	13.8	0.032	4 a	3 a	30 a	12 a	9 a	13 a	10 a
<i>Synanthedon myopaeformis</i>	Total	Poisson	0.422	99.4	<0.001	34 b	62 c	49 bc	–	3 a	8 a	–
<i>Ptycholoma lecheana</i>	Total	Poisson	0.661	7.5	0.024	1 a	2 a	8 a	–	–	–	–
<i>Archips podana</i>	Total	–	–	–	–	–	3	5	–	–	–	–
<i>Hedya nubiferana</i>	Total	–	–	–	–	–	3	5	–	1	–	1
<i>Pammene</i> ssp.	Total	–	–	–	–	–	–	1	–	–	–	1
<i>Pandemis heparana</i>	Total	–	–	–	–	–	–	5	–	–	–	–
Unidentified	Total	Poisson	0.996	5.2	0.518	7 a	8 a	3 a	3 a	3 a	4 a	4 a



**Fig. 1.** Boxplot of female and male CBT caught in transparent delta traps baited with plant volatiles in 2018. Treatments labelled with the same letters are not significantly different (GLM, negative binomial distribution).

not differ from those in unbaited traps. Bycatches of the moth *Anthophila fabriciana* (Linnaeus 1767) (Choreutidae) were scored in the AA/PET/PE, in the LOXP and in the PET/LOXP/PE treatments, while fewer individuals were captured in blank traps and in traps loaded with the other blends (Fig. 2). The same AA/PET/PE blend was capable of capturing the apple clearwing moth *Synanthedon myopaeformis* (Borkhausen 1789) (Sesiidae). Captures of this species were slightly, but not significantly increased by the addition of LOXP to the AA/PET/PE blend, while a lower number of insects were scored in the binary blend of AA/LOXP. Significantly less *S. myopaeformis* were also caught in the PET/PE and the LOXP/PET/PE blends. Although to a much lower extent, the AA/PET/PE (with or without LOXP) blend was behaviourally active also on three tortricid species, i.e. *Archips podana*, *Ptycholoma lecheana* (Linnaeus 1758) and *P. heparana*, and caught significantly more other insects than the other blend except AA/PET/PE/LOXP (Fig. 2).

### 3.2. Seasonal flight and distribution

The seasonal flight recorded by the experimental traps is presented on Fig. 3. In 2018 (Viken) moths were caught since the first week of testing, indicating that the flight might have started earlier (Fig. 3). While two peaks of male activity were observed, most females were captured within a single peak, which corresponded to the later peak of males. In 2019, a higher number of adult CBT and an earlier first catch was recorded in Viken compared to the other trapping station in Hardanger (Figs. 3 and 4). In Viken, the peak in male flight activity occurred in week 25 followed by a peak of females two weeks later. In Hardanger the number of caught moths were much lower, with no clear flight pattern being detected. As registration of trap catches lasted until week 31, no information on activity later in the season is available.

### 3.3. Damage

The damage registered on the tree trunk of different varieties varied from 0 to 91% (Fig. 4). In some replicates, all trees were damaged. The number of damaged trees varied considerably between place and varieties. Although damage was found in all orchards with moth catches, the data did not allow for a correlation between catches and damage, due to a large variation of sensitivity among varieties. In two orchards with no catches in Hardanger we found 1% and 25% damage, respectively. The varieties with the highest number of damaged trunks were Summerred

(91%) and Aroma (25–75%), while Gravenstein and Discovery showed an intermediate (38%) and a low (4%) attack by CBT, respectively (Fig. 4).

## 4. Discussion

In this study we carried out field trapping experiments to measure *E. formosana* attraction to volatile blends of candidate compounds. Blends including AA and LOXP caught the highest number of both sexes. While addition of PET and PE did not significantly change the attraction, a significant reduction of female catches was observed when LOXP was replaced by PET and PE. The first behavioural effect of LOXP as female attractant in the field was recently reported for *C. pomonella*, another tortricid pest which has adapted to feed in the fruit of apple and a few other plants (Knight et al., 2019a). CBT attraction to AA/LOXP blends could be explained by the emission of LOXP by cherry wood (Setzer, 2016). As an alternative, LOXP might be released by microorganisms infecting the plant, such as *Aspergillus niger* (van Tieghem, 1867) (Demyttenaere et al., 1998), which might be involved with moth feeding along with AA.

Only blends including AA were capable of catching CBT in our experiment. AA is reported as a fundamental component of blends attractive for *C. pomonella*, *P. cerasana*, *P. heparana*, *P. limitata*, *P. pyrusana*, *S. ocellana*, *L. botrana*, *A. xylosteana*, *C. rosaceana* and *E. postvittana* (El-Sayed et al., 2016; Judd et al., 2017a, 2017b; Knight et al., 2017; Larsson Herrera et al., 2020b; Tasin et al., 2018). Whereas PET did not increase attraction to AA and LOXP as a minor cherry wood constituent, other main components of fruit tree wood such as benzaldehyde and benzyl alcohol could play a role in enhancing CBT response to the binary blend of AA and LOXP and need to be tested (Setzer, 2016). Furthermore, attraction to AA alone and in combination with additional wood components may also play a role and needs to be tested in further experiments. Male CBT attraction to the ternary blend of AA/PET/PE did not differ from that to the AA/LOXP blend. Such an effect is not surprising, because the generic capacity of this blend to attract several tortricid males, such as *Pandemis* spp., is already reported (Larsson Herrera et al., 2020b). However, we did not find any additive effect by adding LOXP to this 3-component blend. Thus, it seems that in the four component blend of AA/PET/PE/LOXP an alteration of the message occurs, reducing attraction in comparison to AA/LOXP. On the other hand, the AA/PET/PE combination showed a lower specificity than the

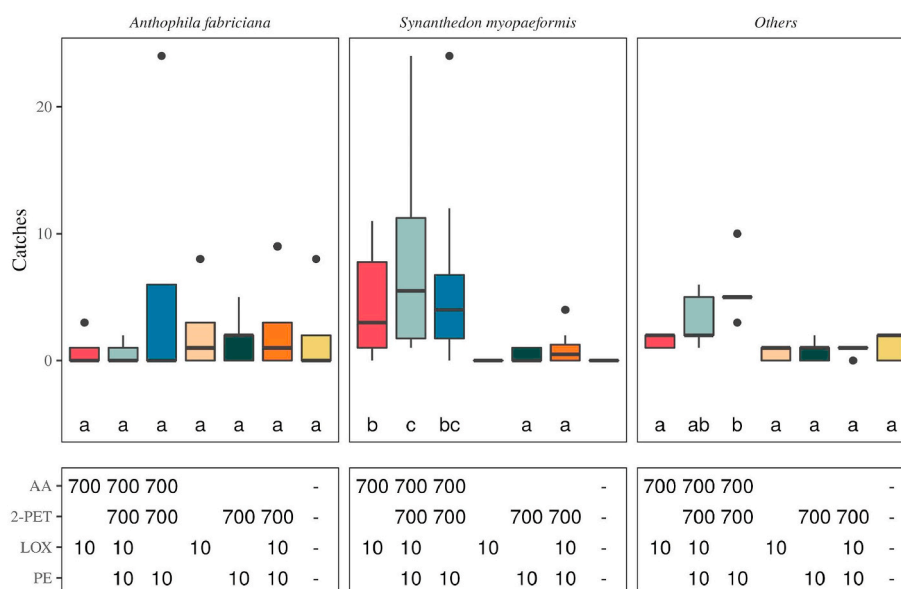
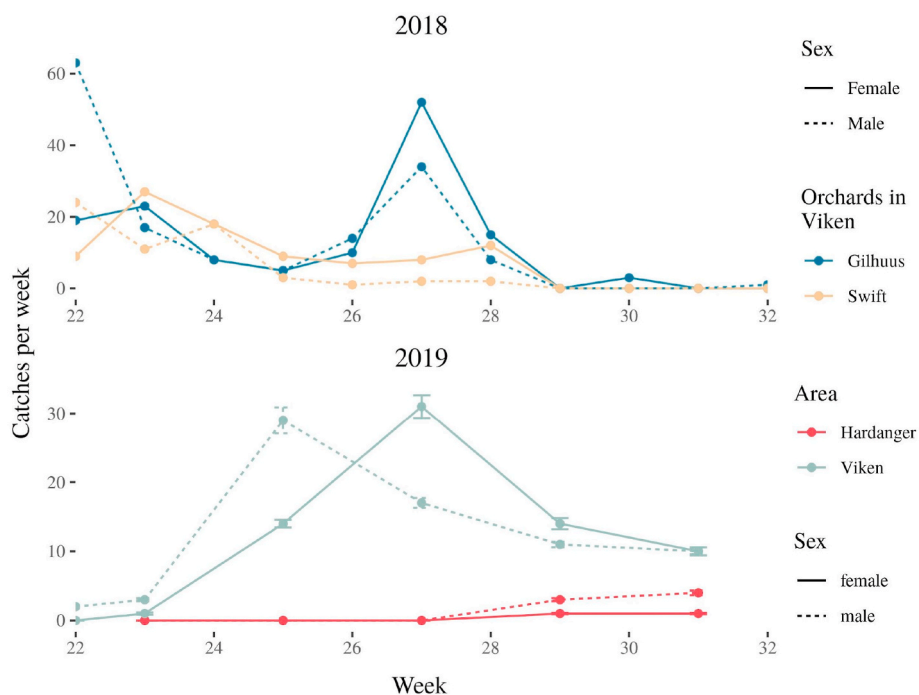
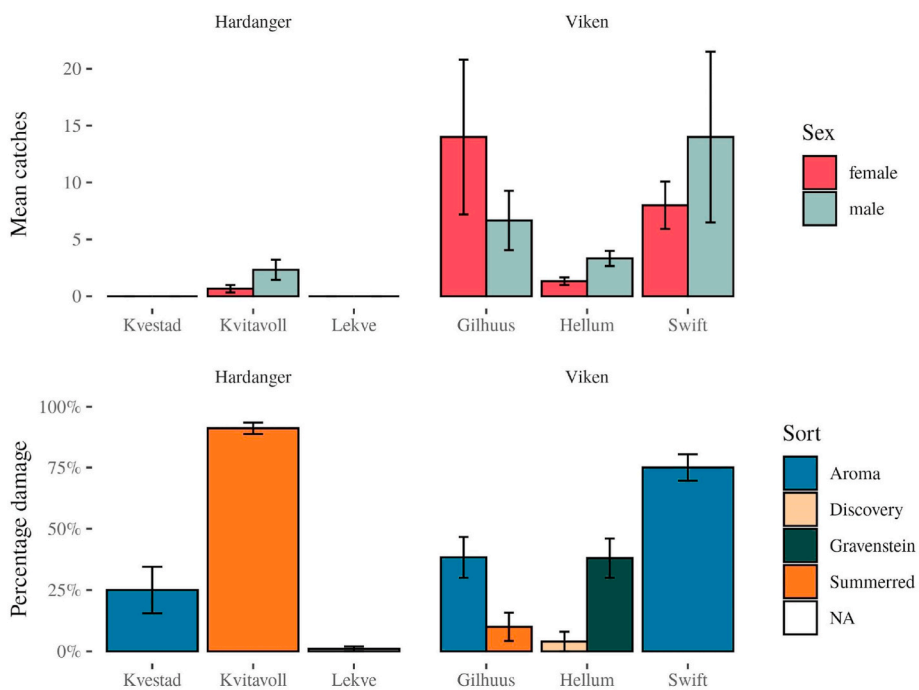


Fig. 2. Boxplot representing bycatches of *A. fabriciana*, *S. myopaeformis* and other Tortricid species in transparent delta traps baited with plant volatiles in 2018. Treatments labelled with the same letters are not significantly different (Glm, Poisson distribution). Treatments with no catch were excluded from the analysis.



**Fig. 3.** Flight curve of *E. formosana* in 2018 and 2019. In 2018 traps were deployed in two orchards in Viken, while in 2019 in 3 orchards in both Hardanger and Viken. Colors represent orchards (2018) or areas (2019). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 4.** (Top) Barplots representing female and male catch in three orchards (x-axis) on two locations (Hardanger and Viken) in 2019. (Bottom) CBT damage on different apple varieties in the same orchards.

AA/LOXP blend, catching additional tortricids (*H. nubiferana*, *Pammene* spp. and *Pandemis* spp.) as well as *A. fabriciana* (Choreutidae) and *S. myopaeformis* (Sesiidae). In general, while the AA/PET/PE with or without LOXP could be used as a generic moth attractant, the AA/LOXP combination shows higher specificity for CBT.

This study provides preliminary information on the seasonal flight of female *E. formosana* and estimated the abundance of this pest in fruit

districts both in Eastern and Western Norway. Due to a long flight period, the number of CBT generations per year has been the object of debate. It has been shown that CBT is univoltine in both USA, Bulgaria and several other European countries (Breedveld and Tanigoshi, 2007; Jenner et al., 2004; Toshova et al., 2017). Our results show a continuous flight activity from early June until early August, supporting the hypothesis that CBT also has one generation in Norway.



In our experiments we found no correlation between the number of insects caught in AA/LOXP traps and larval damage of CBT in the trunk. Factors such as variety susceptibility, tree age and microclimate might have added uncontrolled variation to our dataset and need to be taken into account when attempting such kind of correlation. For example, Dickler et al. (1972) showed that the removal of grass and weeds growing around the trees had an effect on humidity and temperature, and resulted in a lower larval infestation.

The high attraction of both sexes of CBT to the binary blend of AA/LOXP represents a first step towards the identification of a multicomponent kairomone for this pest. In our study, the AA/LOXP blend allowed for the estimation of the number of generations and provides a promising tool for the evaluation of population level across fields and regions. Additional studies with a higher number of data points across varieties and tree age will be necessary to attempt a possible correlation between accumulated trap catches in kairomone traps and trunk damage caused by the next larval generation.

#### CRedit authorship contribution statement

**Gunnhild Jaastad:** Writing - original draft, Writing - review & editing, Methodology, Investigation, Funding acquisition, Project administration. **Sebastian Larsson-Herrera:** Formal analysis, Investigation, Visualization, Writing - review & editing, Methodology. **Marco Tasin:** Writing - original draft, Writing - review & editing, Methodology, Resources, Conceptualization, Funding acquisition, Project administration, Supervision.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgement

MT was supported by the Swedish Farmer's Foundation (project SLF-H2019) and the framework of the ERA-Net C-IPM, with funding from Formas (FP7-KBBE; project API-tree).

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