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The influence of temperature on the synthetic pheromone of the grape moth *Lobesia botrana* (Lepidoptera: Tortricidae) and on pheromone components of three other species of moths

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Rezumat

Influenţa temperaturii asupra feromonului sintetic al moliei strugurilor *Lobesia botrana* (Lepidoptera: Tortricidae) şi a componentelor feromonale ale altor trei specii de molii

Feromonul sintetic al moliei strugurilor *Lobesia botrana* a fost încălzit la 100°C. A fost examinat şi după încălzire prin GC-MS(D), iar atractivitatea sa a fost testată cu capcanele amplasate în câmp. Structura multiplilor produşi de degradare a fost discutată. La fel ca la feromonul pentru *Lobesia botrana*, componentele feromonului sintetic al altor trei specii arată o bună stabilitate în aceleaşi condiţii.

Abstract

The synthetic pheromone of the grape moth *Lobesia botrana* was heated to 100°C. It was examined before and after the heating by means of a GC-MS(D), and tested for its attractivity by field trapping. The structure of several degradation products was discussed. It turned out that oxydative together with thermal processes are involved in the observed pheromone degradation. Unlike the pheromone of *Lobesia botrana*, the synthetic pheromone components of three other moth species showed under the same conditions a good stability.

Keywords: *Lobesia botrana*, *Lymantria dispar*, *Synanthedon myopiformis* and *vespiformis*, *Adoxophyes orana*, Lepidoptera, Tortricidae, sex pheromone, temperature, field traps tests, vineyard

Introduction

The degrading effects of temperature on pheromones with conjugated double bounds have been studied both in field and in laboratory conditions (SHANI & KLUG 1980a, b, SHANI et al. 1982, IDESES et al. 1982a). IDESES et al. (1982a, b) proposed for this proces an oxydation reaction with a radical mechanism, in according to a later work from IDESES & SHANI (1989).

In the present work, the degradation velocity of the conjugated diene pheromone of *Lobesia botrana* and the chemical structure of several degradation products were discussed. Also the comparison of the stability of pheromones with different chemical structures under the same conditions of heating were studied. In addition, the attractivity of the products to moth (*Lobesia botrana*) was studied in the field.

Methods and materials

Pheromones. The following pheromones have been investigated:

· (E,Z)-7,9-dodecadienyl acetate, the main component of the sex pheromone of *Lobesia botrana* (Buser et al. 1974),

· cis-7,8-epoxi-2-methyl octadecane, the main component of the sex pheromone of *Lymantria dispar* (Bierl et al. 1970),

· (Z,Z)-3,13- octadecadienyl acetate, the main component of the sex pheromone of *Synanthedon myopiformis* and *vespiformis* (VOERMAN et al 1983),

· (Z)-9-tetradecyl acetate, the sex pheromone of *Adoxophyes orana* (MEIJER et al. 1972).

The pheromones and the pheromonal baits used in this work were supplied by the Institute of Chemistry „Raluca Ripan" Cluj-Napoca.

Heating. The heat exposing was done at 100°C in 54 glas vials of 2ml with teflon filling lids, hermetically closed, per vial with 1µl of synthetic pheromone (without solvent) of *Lobesia botrana*

species. The tightly closed vials were wrapped in aluminium foil, so that the heating occurred in the dark. The device was a „Termochem-Metallblock" model CHL, Liebisch corporation, type 5100 6201.

During the first 70 min, at every 10 min, three samples were taken out of the heater, and their content of (E,Z)-7,9-12 OAc was measured.

For the field trapping, 8µl per vial of *Lobesia botrana* pheromone were heated under the same conditions for 21 hours.

In order to compare the stability of the pheromone of *Lobesia botrana* to the pheromones of the other three species, these too, were heated at 100°C in the dark, in the same type of vials. The testing was done during the 60 hours as follows: the first 24 hours at every 6 hours, then at every 18 hours. On this purpose, 21 vials, each supplied with 1µl pheromone, were prepared, and three vials were tested each time.

GC-MS. Before and after heating, each probe was investigated using a gas chromatograph connected with a mass-spectrometer, model HP-5890II/ 5972. The gas chromatograph had a HP-5MS column 30 metres long, with a diameter of 0.25mm, and a film thickness of 0.25µm. The method applied allowed to record masses up to 350 units. Two temperature programs were used (Tab. 1):

At the beginning, the temperature was 100°C and the initial time was 2 min. The injection of the samples was automatic, the solvent used was n-hexane. The second program was used in order to make a better separation of some degradation products.

Traps. Adhesive traps of a Delta type were used, delivered by the W. Neudorff GmbH KG corporation, 31860 Emmerthal, Germany.

The field tests were made within the period from July, 25 ab to August, 23, 1998.

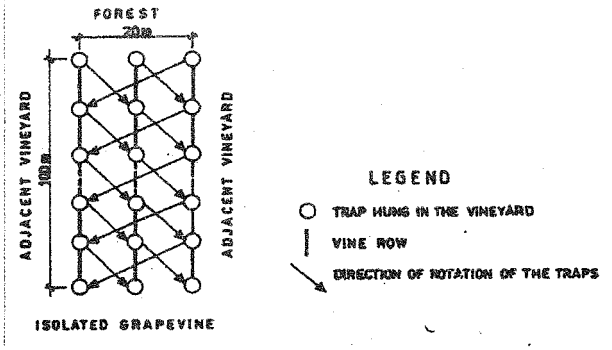


Fig. 1. The vineyard and the rotation of the traps

In the vineyard 16 traps were hung, each of them baited with 1µl of the *Lobesia botrana* pheromone as follows:

- 8 traps, containing the fresh pheromone,
- 8 traps, containing the heated pheromone.

The traps were hung in a vineyard cultivated according to biological methods, about 2000m, large, where no insecticide or herbicide were used.

The distance between traps was 10-15 m. This vineyard is located between other vineyards.

At every three days the position of the traps were changed as shown in Fig. 1.

Table 1.

The temperature programs used in the GC-MS analysis

Level	Rate (°C/min)		Final temperature (°C)		Final time (min)	
	Program 1	Program 2	Program 1	Program 2	Program 1	Program 2
1.	40.00	60.00	140	170	0.00	0.00
2.	5.00	3.00	220	190	0.00	0.00
3.	60.00	60.00	280	280	2.00	2.00

Results and discussions

Labor trials

The (E,Z)-7,9-12 OAc concentration decreased to zero after 70 min of exposing at 100°C (Fig. 2). During the exposure a degradation product appeared, which had almost the same time of retention as the (E,Z)-7,9-12 OAc. For a better separation of the products, temperature program 2 was used (Tab. 1).

The control sample of the pheromone of the grape moth *Lobesia botrana* shows the (E,Z)-7,9-

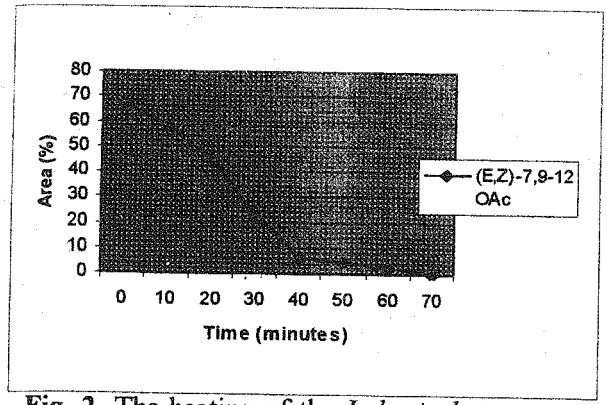


Fig. 2. The heating of the *Lobesia botrana* pheromone at 100°C in the dark for 70 min. The data represent average values obtained from three measurements

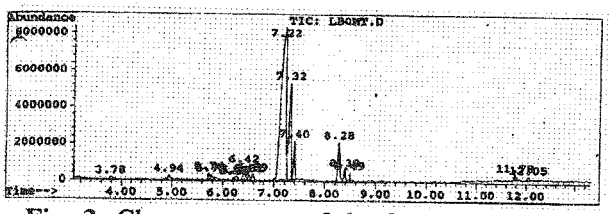


Fig. 3. Chromatogram of the *Lobesia botrana* pheromone, control sample of fresh pheromone

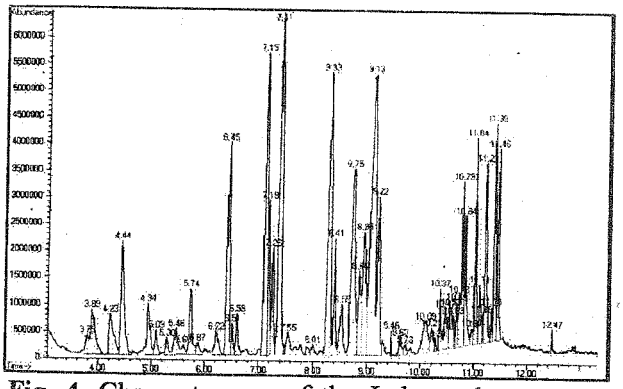


Fig. 4. Chromatogram of the *Lobesia botrana* pheromone, sample after 40 min heating at 100°C in the dark

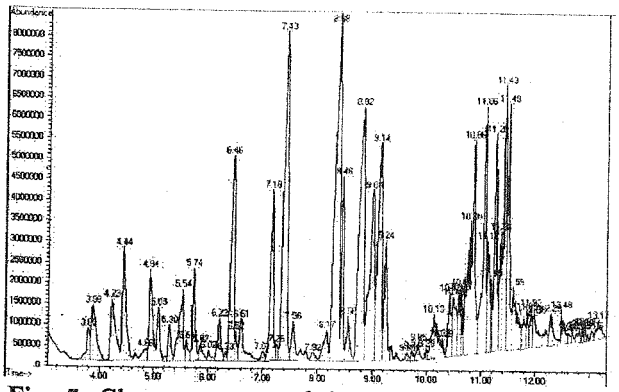


Fig. 5. Chromatogram of the *Lobesia botrana* pheromone, sample after 70 min heating at 100°C in the dark

12 OAc peak at 7.22 min (Fig. 3). After heat exposure for 40 min, the peak of this compound appears at 7.15 min, together with a peak at 7.19 min which corresponds to a newly formed degradation product

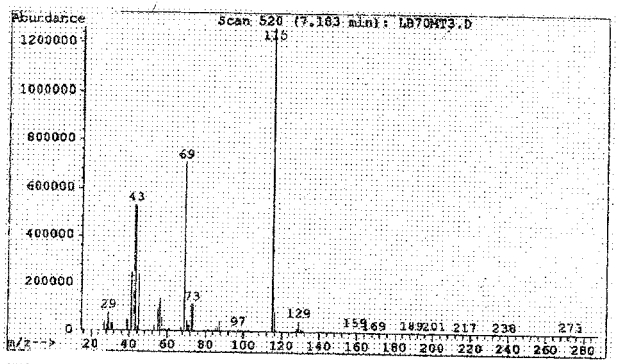


Fig. 6. The mass-spectrum of the peak at 7.18 min from Fig. 5

(Fig. 4). Fig. 5 shows the sample exposed to heat for

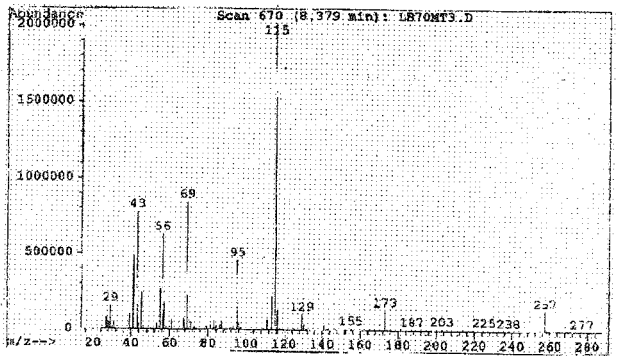


Fig. 7. The mass-spectrum of the peak at 8.38 min from Fig. 5

70 min: the peak of the new substance appears at 7.18 min, the (E,Z)-7,9-12 OAc has been completely degraded. The concentration of the new product (retention time 7.13 ± 0.10 min)* increases according to the decreasing concentration of (E,Z)-7,9-12 OAc (retention time 7.12 ± 0.13 min)* and arrives an average value of 2,86%. (*average values of all measurements from Fig.2)

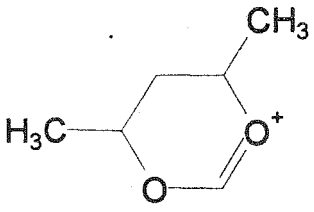
In Fig. 5, the peak at 8.38 min has a mass-spectrum similar to the peak at 7.18 min, with an ion of maximum intensity at m/z 115, followed by ions at m/z 69 and m/z 43 (Figures 6 and 7).

The main peak m/z 115 suggests a structure that contains an oxygen atom. The ion (*b*) below is two mass units bigger than its hydrocarbon homologue with eight carbon atoms, (*a*). Another possible structure could be with two oxygen atoms in the molecule and an unsaturations, the ion (*c*), or with two oxygen atoms and two unsaturation, like the 1,3 dioxane ion (*d*) and the peroxide ion (*e*):

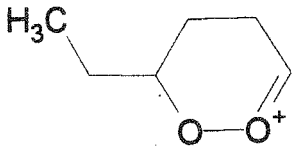
a, m/z 113

b, m/z 115

c, m/z 115



d, m/z 115



e, m/z 115

Fig. 8 presents the mass-spectrum of the product with the retention time 7.18 min (Fig. 5) and the mass-spectrum of cis-2, 4, 6-trimetyl-1,3-dioxane, found from the Library Wiley 275, as the most probable structure. For comparison, the structure of the ion m/z 115 was written as a peroxide (*e*) which seems more likely if one considers the structure of the initial pheromone.

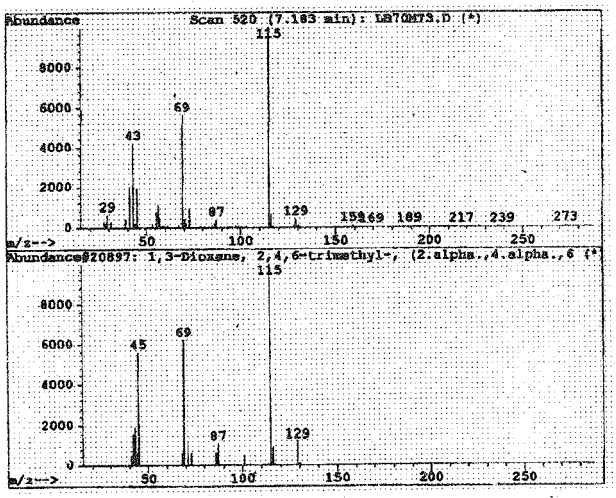
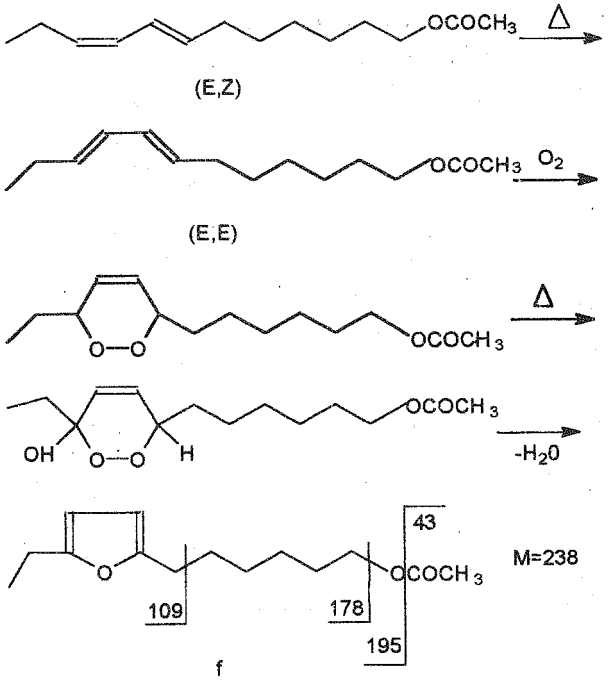


Fig. 8. Comparison of the mass-spectra of the product with a retention time of 7.18 min in Fig. 5 (top panel) and of cis-2, 4, 6-trimetyl-1,3-dioxane (bottom panel)

The degradation product with a retention time of 7.43 min (Fig. 5) is most likely an alkylfuran with the (*f*) structure (shown below). The chemical reactions leading to its formation can written by analogy to the researches of SHANI & KLUG (1980a) and IDESE et al. (1982b):



The structure of the alkylfuran can be easily recognized from the peak at m/z 109 of its mass-spectrum (Fig. 9) as reported by SHANI & KLUG (1980a) from the main component of the sex pheromone of *Spodoptera littoralis* (Z,E)-9,11-14 OAc. This ion forms up through a split in a benzyl position (HESSE et al. 1984).

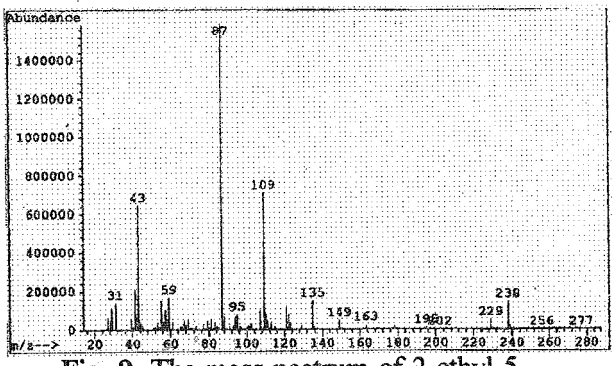


Fig. 9. The mass-pectrum of 2-ethyl-5-acethylhexyl-furan, a degradation product of the *Lobesia botrana* pheromone, after heating to100°C in the dark

Other peaks of smaller intensity (Fig. 5) will be analysed in the following.

The mass-spectrum of the peak at 4.44 min (Fig. 5) is presented in Fig. 10.

This substance might be an acetate with M=186. The main peak m/z 43, corresponding to the ion $CH_3—C\equiv\overset{\oplus}{O}$ (OPREAN 1974), together with the ion m/z 61 proved the acetate structure of this substance. The ions being present in this mass-spectrum with higher mass-values than 186 (m/z 207 and m/z 341) might be consider impurities.

The peak at 4.94 min (Fig. 5) is most likely the 7-decenylacetate with M=198, found from the Spectrum Library Wiley 275, as the most probable structure (91% similarity). This can be observed in

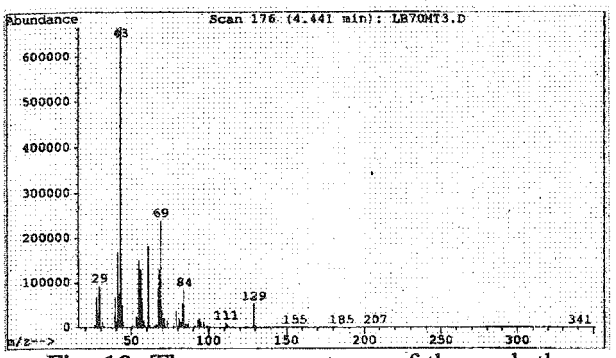


Fig. 10. The mass-spectrum of the peak the retention time of 4.44 min in Fig. 5.

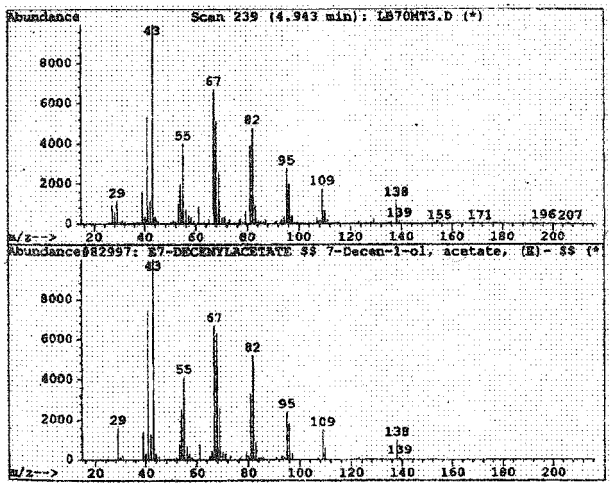


Fig. 11. Mass-spectrum of the component with a retention time of 4.94 min in Fig. 5 (top panel) and of (E)-7-decenylacetate (bottom panel)

the Fig. 11. The m/z 138 corresponding to an ion after losing a neutral molecule of acetic acid, through a McLafferty transposition (BUDZIKIEWICZ 1992), indicates a typical fragmentation for acetates.

The mass-spectrum of the peak at 6.47 min (Fig. 5) presents all the ions of the (E)-7-decenylacetate with M=198 (proposed by soft) together with other ions. It would be possible, that this substance have an acetate structure with a chain with 10 or more carbon atoms and one unsaturation.

Comparing the ions of the peak at 9.18 min (Fig. 5) with those of the 2, 2, 4-trimethyl-2,5-dihydrofuran, M=112, proposed by the software, it can be observed that all the ions of the furanic structure are also present in the mass-spectrum of the searched substances. Beside those, other heavier ions appear, the last one recorded at m/z 277. Considering the initial structure of the grape moth pheromone it is supposed that the searched product is 2, 5-dihydro-alkylfuran.

For comparison, pheromone components of three other moth species were heated under the same conditions. The pheromone of *Adoxophyes orana* has the structure of a monoenic acetate with the double bound in the position 9. The pheromone of *Synanthedon myopiformis* and *vespiformis* is also an acetate with two isolated double bonds, position 3 and 13, and the main component of the *Lymantria dispar* pheromone is an epoxide. All three structures

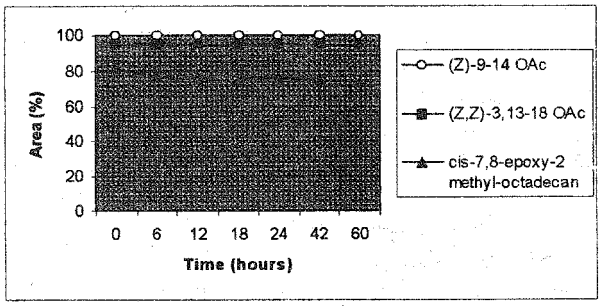


Fig. 12. Relative amounts of three pheromone components after exposure at 100°C in the dark for up to 60 hours

show a high stability in time (Fig. 12).

Heating in the dark of the cyclohexane solution of the main component of the female pheromone of the Egyptian worm of the cotton leaf *Spodoptera littoralis*, (Z,E)-9,11-tetradecadienyl acetate, at 50°C in the absence of air during a few weeks, does produce neither oxidation nor izomerization (SHANI & KLUG 1980b). The same authors notice that heating of the pheromone without solvent during 2-4 days triggers the complete decomposition, resulting in a unidentifiable resin residue.

In our case, the (Z,E)-7,9-12 OAc was complete degradated after 70 min of heating at 100°C. This might be explained by the fact that in our case the pheromone was heated without solvent, and the vials contained two ml of air. Thus, inside the vial oxidation could take place.

We can conclude that the oxidation plays a decisive role especially for the degradation of the conjugated diene pheromones, even when the quantity of oxygen is small. Obviously the oxidation was accelerated by the high temperature.

It remains an open question of whether besides oxidation there was a thermal degradation of the pheromone. Certainly the quantity of air related to that of pheromone plays an essential role in the degree of degradation. The degradation is promoted by a larger relative amount of oxygen and also by a higher

temperature. The nature of the degradation products (decenylacetate, endoperoxide, alkylfuran, 2,5-dihydro-alkylfuran) supports the hypothesis of the thermal and oxidative degradation processes. Furthermore, the experiments presented here demonstrate that the conjugated dienes were much faster degraded that the monoene compound, the diene structure with isolated double bonds and the epoxide. Thus, a decisive role is played by the structure of the pheromone in question.

Vineyard experiments

After heating in the dark for 21 hours, the component (E,Z)-7,9-12 OAc of the *Lobesia botrana* pheromone was still the dominant component, 23% of the initial amount were still present according to GC tests.

Throughout the period of the field experiments, grape moth were observed in the vineyard. In traps containing baits with the fresh pheromone 92 males of *Lobesia botrana* were caught within 29 days. The traps baited with the heated pheromone samples, caught 22 males of *Lobesia botrana* (24 % compared to the control), 3 males of *Cydia pomonella*, 1 male of *Lymantria dispar* and 22 insects of other species.

The heated pheromone samples used for the field tests were degraded much more slowly (77% within 21 hours) compared with previous experiments shown in Fig. 2 (almost 100% degraded within one hour). The reason could be that in the previous experiments the glass vials exposed to the heat were loaded with only one µl of pheromone whereas they contained eight µl in the field experiments. We can conclude that the 2 ml of air, respectively their content of oxygen, present in the glass vials, fully oxidized the small amount of pheromone but were not sufficient to oxidize the larger amount.

It is supposed that the velocity of degradation would have been rather constant if the exposure was continued after the oxygen in the vials was used up.

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