

A Preliminary Study on the Simulation Model for Determining the Absorption of Pheromone of *Lobesia botrana* Den.-Schiff. by Grape Leaves

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Introduction

Viticulture is an important agricultural activity with an acreage of 160 thousand hectares of growing area in western the Aegean Region in Turkey (Çetin and Tipi, 1998). The European grapevine moth (*Lobesia botrana* Den.-Schiff.) is the key pest of grapevine in Turkey and more generally in Europe. It produces 3-4 generations per year in Turkey. The damage caused by larvae of the third generation is the most serious, because the larvae destroy the berries directly (Altindi_li and Kışmalı, 1998). The mating disruption technique with pheromones was first put into practice in 1999 to control the grapevine moth (Altindi_li et al., unpublished data). Isonet-L dispensers possessing 172 mg pheromone each were evaluated as effective to control *L. botrana*, as the unique method. No insecticide application was made against *L. botrana* in the vineyards where 600-650 Isonet-L dispensers/ha had been installed. Seedless grape (*Vitis vinifera* cv. Sultanina) is the most common variety in the region. It is pruned as long and short canes. The vegetative growth of the variety is also more vigorous than those of wine and table varieties. Wall, big T and Y are the most popular trellis systems. Until recently, wall and big T were the most wide spread training systems. The preference varied according to the regions. Lately the newly established vineyards are trained as Y. Each system has different leaf density and leaf area. Sometimes, the efficacy of mating disruption technique varies year to year or is not stable throughout the season. Moschos et al. (1998) expressed that unsatisfactory results of mating disruption technique may be attributed to different factors such as the high population density which increases the chances of mating, the effects of prevailing high temperatures and strong winds on the concentration of pheromone in the vineyard and may be the low or uneven distribution of the pheromone which might have been inadequate to compete with the natural pheromones of the females in the vineyard. For good results it must be used in large areas or in sufficiently isolated vineyards so as to reduce as much as possible the danger of mated females coming in from neighbouring zones (Ogawa, 1997; Casagrande and Jones, 1997). Another important factor is the leaf area during critical periods in terms of target pest. Providing an adequate release rate for an application during spring is especially important. Due to sparse foliage, pheromone can easily disperse in atmosphere. Schmitz (1997) and Neumann (1996) reported that the leaves could absorb and release the pheromone to the atmosphere again. In addition, the effect of wind increases due to poorer foliage of fruit or vine trees in spring. Accordingly, pheromone concentration, which was not absorbed by leaves, can easily disperse in atmosphere (Neumann, 1996). In a pioneer study in Turkey, the confusion effect of pheromone absorbed by leaves in pheromone-treated vineyards continued until the harvest at the end of August even if the pheromone in the dispensers seemed to be consumed by July 11, 2000 according to the estimation of release rate. On the other hand, the pheromone amount seemed to be consumed on August 3, 2001 according to weekly weight loss in the same study (Altindisli et al., unpublished data). Because of the larger leaf area and density of Seedless Sultanina, the presence and quantity of pheromone in grape leaves have great importance. Leaf density and

leaf area of Sultanina can play an important role on the efficacy of mating disruption technique. It was aimed to clarify the absorption and desorption of pheromone and the role of different leaf densities in the three important trellising systems in the Region.

Material and Method

Simulation Study

Exposure of leaves to pheromone:

Application of the standard: In the first step, 8 untreated mature leaves 12 cm in diameter were put into glass jars. E-7, Z-9, dodecadienyl acetate was added into the jars by using 'Transferpette', automatic micropipette. Pheromone was not applied onto the leaves, directly. Pheromone doses applied were 10, 50, 250 and 1000 ml as four replicates. After the application, parafilm was used to cover the jars to prevent gas release. The leaves were kept for 4 days with pheromone at 24-26 °C, 16: 8 LD.

Absorption of pheromone by active charcoal:

After 4 days, these leaves were transferred to clean glass jars with 0,2 mg active charcoal in porcelain cups. Parafilm was used to cover the jars to prevent gas release. The leaves were enclosed in jars with charcoal for 2 days before GC analysis.

Extraction and analysis of pheromone:

Charcoals were eluted in ultrasonic bath with 10 ml of n-hexane for 3 minutes. After the elution, the samples were evaporated to dryness under nitrogen. 0,5 ml of n-hexane was added to prepare the samples for GC analysis. For the determination of pheromone, HP 6890 GC equipped with HP 5973 Mass selective detector was used. All injections were performed by Agilent 6890 Series Injector.

Comparing pheromone levels in three different trellis systems:

Three experimental fields were chosen for sampling from pheromone-treated vineyards trained as T, Y and Wall trellis systems in Manisa Province in the Aegean Region. Isonet- L dispensers containing 172 mg E-7, Z-9 dodecadienyl acetate in each were hanged on 24 April 2002 to control *L. botrana* at a density of 24 m² /dispenser in the center and at 2 m intervals on the borders (616 dispensers/ha. On 14 August 2002, 40 leaves near the dispensers were collected from each trellis system. The samples were kept in the freezer at -18 °C until the analysis.

After sampling, 10 leaves were put into each glass jar with 0,2 mg active charcoal in porcelain cups as four replicates. Parafilm were used on top to prevent gas release. They were maintained for 5 days at 24-26 °C, 16: 8 LD. The procedure explained in extraction and analysis of pheromone was followed to prepare the samples for analysis. Quantification is done by GC.

Results and Discussion

In this simulation model, gas exchange conditions of grape leaves were simulated in standard volume gas-tight jars. Active charcoal method, which was used for determining the quantity of pheromone in a treated field atmosphere in previous studies, was modified and applied in this study. Pheromone applied into the jar was absorbed by grape leaves and captured in active charcoal as the second step. Following this procedure, grape leaves from treated vineyards were analysed to determine the desorption of field-applied pheromone.

The results obtained in simulation model showed that grape leaves may absorb and desorb pheromone at different levels both under field and controlled conditions. A calibration curve was plotted between injected pheromone levels of 10, 50, 250 and 1000 ml and signals

obtained by GC-MS. The correlation coefficient (R^2) was 0,9776, equation was $y=19,233x+5076,1$.

T, Y and Wall trellis systems were compared according to the fragment at 20.24 minutes (121, 138, 155, 166, 194 ions) in terms of desorption of pheromone from the grape leaves. The pheromone intensities were 30400, 22000 and 18800 in T, Y and Wall trellis systems, respectively. The highest desorption level was seen on T system. The lowest desorption level was found on the samples from Wall system. This can be attributed to stronger air circulation inside of the canopy in the Wall system, which has lower leaf area and density than the other two systems.

Leaf removal, which is one of the summer pruning practices in round seedless variety in the Aegean Region in Turkey, is an important cultural measure. A great number of mature leaves are removed around bunches by cutting to speed up maturation of grapes and canes, to improve colour formation on berries and to decrease humidity in the canopy. This process may coincide with some biological development stages of *Lobesia botrana* such as copulating, egg laying or hatching which are critical in terms of efficiency against the second and third generations. Heavy leaf removal in the course of these critical periods may cause loss of grape leaves which absorb pheromone and will release and work as a dispenser. Moreover, stronger air circulation formed in the canopy disperses pheromone in the atmosphere. Consequently, the efficiency of mating disruption technique may decrease if the leaf removal is carried out as such.

On the basis of the results obtained after 5 days of keeping in jars, the grape leaves may carry over pheromone at different concentration levels depending on the systems. Desorption rate of the leaves may be accounted for the accumulation of pheromone at different concentrations. This study will be followed by a solid phase extraction (SPE) system in order to obtain the total concentration level of pheromone on the leaves. The recovery of the system will be calculated after this simulated study is completed. Accordingly, change in timing of leaf removal in practice for the Aegean Region will be more important regarding the critical periods of the second and third generations of the pest such as mating, egg laying and hatching. Keeping mature leaves during the critical periods will support retaining of pheromone on canopy.

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