SELECTED OF SEEDLINGS OF \textit{THYMUS VULGARIS} BY GRAZING SLUGS

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SUMMARY

(1) An experiment is described which tests the effects of different terpene compositions of plants of \textit{Thymus vulgaris} on their palatability to slugs.

(2) The results are analysed using a Markovian model. They show that the different terpene compositions provide very different defences against the slugs and that the behaviour of an individual slug is determined by several factors including the past experience of the animal.

INTRODUCTION

Predation is one of the most difficult problems plants encounter because the defences of the plant may be made ineffective by a change in the capability of the predator. Therefore, the genes concerned with this kind of adaptation are very likely to be polymorphic.

Many species of plants produce a lot of seeds per individual, and most of these do not give rise to an adult as they are eliminated at the seedling stage. At this stage, predation is an important demographic factor because (i) the size of the plant is small so that a herbivore can easily destroy it entirely and (ii) young individuals are more heavily grazed than old ones are (Coley 1980). Any individual which has a defence against herbivores at this stage might be expected to have a very powerful selective advantage.

This is particularly true for a perennial woody species such as thyme (\textit{Thymus vulgaris} L.). This species was shown to produce a variety of terpenes which differ from one individual to another (Passet 1971). The terpenes are mainly in the leaves. They are produced in special cells which store them in vacuoles. These cells are epidermal, are broken easily and the terpene is then released into the atmosphere giving the plant its well-known scent. Because they are found mainly in the leaves and throughout the year the terpenes are very unlikely to play a role in pollination. These characteristics made us think that the terpenes might be able to deter the slugs from eating the plants. Such an effect has been found in the perennial mint \textit{Satureja douglasii} (Rice, Lincoln & Langenheim 1978; Lincoln & Langenheim 1979) and the terpenes of thyme have been shown to influence the behaviour of ants (Jaisson 1980). Here we record the results of an experiment to test the effect of the different terpene compositions on a predator of the plant.

MATERIAL AND METHODS

The terpene composition of plants of \textit{Thymus vulgaris} differs from one plant to the other (Granger, Passet & Verdier 1963; Passet 1971) and has been shown to be governed by five or six genetic loci (Vernet 1977; Vernet, Guillerm & Gouyon 1977a, b; Gouyon &
Vernet 1980). The structure of the terpenes is shown in Fig. 1. In one individual, one can find only one of the six terpenes shown; the six plant chemotypes will be designated by the appropriate letter.

In the present study, only types A, U, C and T will be considered; G was not used because it is rare in natural populations and hence it is difficult to obtain a sample of seeds which contains only this type; L was not used because it behaves in a peculiar way. When the L individuals are young, they produce a phenolic compound (T or C according to the genotype of the plant). Only when they are 2 or 3 months old do they block this synthesis and produce linalool.

The slug Agriolimax reticulatus (Muller) was chosen for the experiment because, during the previous year, some young plants had been seriously damaged by them in our glasshouse near Montpellier. These slugs (1–3 cm long) were common in the grass growing around the glasshouse. The day before the beginning of the experiment reported here, some black plant pots were placed upside-down in the grass and the slugs which moved into them were collected the following morning. These slugs had probably eaten just before being caught but they had probably never eaten thyme because it does not grow outside in the vicinity of the glasshouses, and the glasshouses themselves were protected with helicides.

The distribution of the chemotypes had been mapped in the neighbourhood of Montpellier. Seeds of thyme were harvested from four populations located in four homogeneous areas of the map containing respectively the A, U, C and T chemotypes. Three hundred seeds from each sample were analysed using an automatic vapour phase chromatograph (Gouyon et al. 1981) to be sure of the homogeneity of the samples.

Ten plastic boxes (17 × 10 × 4 cm deep) hermetically closed at their base and which could be covered with a mesh lid were filled with sterile soil (60% lime, 40% sand). Thirty-two seeds of thyme were sown in each box (eight seeds per chemotype) according to the regular pattern shown in Fig. 2, so that each type had an equal chance of being grazed. Some seeds did not germinate and some seedlings were removed because they were

![Fig. 1. Formulae of the monoterpenes of Thymus vulgaris (Passer 1971).](image)

![Fig. 2. Arrangement of the four chemotypes of Thymus vulgaris in the experiment. A, U, C and T refer to the terpenes whose structure is shown in Fig. 1.](image)
not at the right stage so that the final number of seedlings was 247. When the plants reached the four leaf stage (actually two leaves and two cotyledons which are leaf-like), one slug was introduced into each box.

The mesh lid was placed over each box and the boxes were placed in the glasshouse. During the day, the boxes were shaded using a board which was placed horizontally, 3 cm above the lids; the slugs nevertheless stayed in a corner of the box all day long. During the night, the boxes were in complete darkness and the slugs were obviously active, as could be seen the following morning from the marks they left on the soil in the whole box. Every morning, each plant was observed and the number of its leaves which had disappeared during the night was recorded. No difference between the results of the different boxes was detected ($P(\chi^2) > 0.40$ for the final results on leaves) so that these results are given for the ten boxes together.

During the first 3 days, the slugs ate respectively 192, 205 and 128 leaves; they were therefore fairly hungry throughout the 3 days. The decrease on the third day may be related to the fact that, at that time, approximately half of the leaves had been eaten and that the choice for the slug was substantially reduced. The experiment was then stopped.

RESULTS

The order of palatability to slugs was $A = C > T > U$ (Table 1). However, this analysis considers that the four individual leaves of each plant are grazed independently. Analyses of the results for individual plants (Table 2) also show that $A$ is the most damaged and that the $U$ type is significantly less frequently killed.

Thus the slugs appear to distinguish plants which are known to differ in the type of terpene they contain. It is necessary for humans to crush the leaves to smell the terpenes; perhaps the slugs also have to damage the plant to distinguish between the chemotypes. To test whether this is true or not, a model which allows description of the behaviour of the slug was constructed.

<table>
<thead>
<tr>
<th>Chemotype of leaf</th>
<th>A</th>
<th>U</th>
<th>C</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grazed</td>
<td>179</td>
<td>90</td>
<td>148</td>
<td>108</td>
</tr>
<tr>
<td>Not grazed</td>
<td>93</td>
<td>174</td>
<td>88</td>
<td>108</td>
</tr>
<tr>
<td>Total</td>
<td>272</td>
<td>264</td>
<td>236</td>
<td>216</td>
</tr>
<tr>
<td>Grazed (%)</td>
<td>66</td>
<td>34</td>
<td>63</td>
<td>50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemotype</th>
<th>A</th>
<th>U</th>
<th>C</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alive</td>
<td>36</td>
<td>55</td>
<td>35</td>
<td>39</td>
</tr>
<tr>
<td>Dead</td>
<td>32</td>
<td>11</td>
<td>24</td>
<td>15</td>
</tr>
</tbody>
</table>
THE MODEL

The behaviour of the slug is assumed to be described by two parameters: the probability of attacking a given plant, \( p_0 \) (related to odour) and the probability of continuing to eat an attacked plant, \( p_1 \) (related to taste). A scheme of slug behaviour described with these assumptions is shown in Fig. 3. At any stage of the experiment, the population of plants with a given chemotype can be described by the number of plants with 0, 1, 2, 3 and 4 eaten leaves.

**Fig. 3.** A model of the behaviour of a slug approaching an undamaged plant.

**Table 3.** State of the individuals of the different chemotypes of *Thymus vulgaris*, subject to grazing by slugs, on each day. The structure of the terpenes A, U, C and T is shown in Fig. 1. (a) Observed values. (b) Computed values assuming that \( p_0 \) and \( p_1 \) are constant in the experiment. The parameter \( p_0 \) is the probability that a slug will attack a plant, and is related to odour; \( p_1 \) is the probability that a slug will continue to feed on an attacked plant, and is related to taste.

<table>
<thead>
<tr>
<th>Chemotype</th>
<th>Day</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>68</td>
<td>47</td>
<td>22</td>
<td>12</td>
<td>66</td>
<td>47</td>
<td>36</td>
<td>28</td>
<td>59</td>
<td>38</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>U</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Number of eaten leaves**
- **(a)**
  - 0: 68, 47, 22, 12, 66, 47, 36, 28, 59, 38, 17, 11, 54
  - 1: 0, 4, 6, 7, 0, 5, 11, 13, 0, 7, 11, 7, 0, 6, 18, 10
  - 2: 0, 7, 9, 7, 0, 5, 6, 9, 0, 4, 5, 6, 0, 2, 5, 10
  - 3: 0, 4, 10, 10, 0, 2, 4, 5, 0, 3, 10, 11, 0, 1, 3, 6
  - 4: 0, 6, 21, 32, 0, 7, 9, 11, 0, 7, 16, 24, 0, 6, 9, 15
  - 0: 68, 40, 24, 14, 66, 49, 37, 27, 59, 34, 20, 11, 54, 35, 22, 14

- **(b)**
  - 0: 68, 40, 24, 14, 66, 49, 37, 27, 59, 34, 20, 11, 54, 35, 22, 14
  - 1: 0, 8, 10, 8, 0, 7, 10, 11, 0, 9, 10, 9, 0, 8, 11, 10

**Estimates of**
- \( p_0 \): 0.41, 0.26, 0.42, 0.36
- \( p_1 \): 0.71, 0.61, 0.66, 0.58
leaves eaten. Each day (j) was associated with a column vector E(j) containing these numbers. The evolution of the plant population can be described as a Markovian process where the state of the population at time j is given by the state at time \( j - 1 \) and a transition matrix \( P_j \) (see Appendix).

\[
E(j) = P_j \cdot E(j - 1)
\]

The experimental results give the values of \( E(0) \), \( E(1) \), \( E(2) \) and \( E(3) \) (Table 3(a)). Assuming that \( P_j \) is independent of \( j \), it is possible to estimate \( p_0 \) and \( p_1 \) over the whole experiment by a maximum likelihood analysis. Table 3(b) shows the result of these calculations which suggest that the model is able to explain the observed data fairly well. The two parameters \( p_0 \) and \( p_1 \) can then be considered to describe the behaviour of the slug adequately.

From these results, it is possible to be more precise about the previous conclusion: \( U \) is less frequently killed because it is attacked less (\( p_0 = 0.26 \)) than the other chemotypes (\( p_0 = 0.41, 0.43 \) and 0.36). It then follows that the slug knows the palatability of the plant without tasting it. The estimates of \( p_1 \) show protection by \( U \) and \( T \) at the level of taste.

The above calculations assume that \( P_j \) is independent of \( j \) (i.e. that the behaviour of the slug is constant). If one does not assume this, it is possible to study the changes in values of \( p_0 \) and \( p_1 \) with time (Fig. 4). These results show that, during the first day of the experiment, the slug did not show a preference for any chemical type according either to taste or to smell. These results are remarkably independent of the chemotype. On the second day, however, the results clearly show a distinction among the chemotypes both by smell and taste. The smell of \( U \) is less attractive to slugs than is that of \( A, C \) and \( T \), whereas the taste of \( T \) and \( U \) is less attractive than that of \( A \) and \( C \).

![Fig. 4. Trends, for the four chemotypes of Thymus vulgaris, of (a) the probability that a slug will attack a plant, \( p_0 \), and (b) the probability that it will continue to eat the plant, \( p_1 \). The structure of the terpenes, \( A, U, C \) and \( T \) is shown in Fig. 1. See text for details.](image-url)
Two caveats must be made. The estimates of \( p_0 \) and \( p_1 \) follow unknown laws and we cannot calculate confidence intervals around them. In fact, we do not need these intervals because the similarity of the estimates on day 1 need not be tested (obviously no test would find a difference among these estimates) and the significant results shown in Tables 1 and 2 prove that there must be a difference sometimes (thus in days 2 or 3). (ii) On day 3, the estimates seem to converge again, although we cannot test this conclusion statistically. This may be due to the restricted choice offered to the animals by that time.

**DISCUSSION**

The results of these experiments, as analysed by the model, indicate that the slugs have, at first, no preference for any terpene chemotype. When they have experienced the taste, however, they are able to learn very rapidly which one they prefer. Rapid learning in slugs has previously been reported (Gelperin 1975; Sahley, Gelperin & Rudy 1981). We do not know the physiological basis of this choice.

Slugs are sometimes able to recognize a terpene compound they do not like without tasting the leaves even though this compound is contained in special glands (as in the U type). By contrast, the T type could not be recognized without tasting. This may be related to the fact that U has a quite peculiar smell while C and T are very similar, at least to the human nose. However, as pointed out by Harper (1977, p. 415), ‘Simply to taste nasty is a poor defence against predators unless the predator can learn to avoid the plants that taste bad’. It seems, in this case, that U is more repulsive than T and Table 1 shows that the protection is really stronger for U than for T.

Molluscan predators are probably among the most important selective factors operating on thyme and one of them is now shown to be able to favour one chemotype. However the system is complicated because the slug does not necessarily have the same behaviour after experiencing some chemotypes. Further experiments, and particularly field experiments, will be necessary to see to what extent the behaviour of predators is responsible for the ecological distribution of the various chemotypes of thyme.

**ACKNOWLEDGMENTS**

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**REFERENCES**


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**APPENDIX**

In the test, it was assumed that the evolution of vector E(j) (describing the state of the plants at time j) would follow a Markovian process (Girault 1954) with a transition matrix Pj. That is, given the vector E(j − 1), the vector E(j) can be calculated by

\[ E(j) = Pj \cdot E(j - 1) \]

In matrix Pj, the element in row i and column k is p(i, k) and corresponds to the probability that a plant having k leaves at day j − 1 will have i leaves at time j. We also know: (i) that p(i, k) = 0 when k > i because plants did not grow during the experiment; (ii) and that \( \sum_k p(i, k) = 1 \) as in any Markovian process.

The assumption of the model is that Pj can be calculated as a function of two parameters \( p_0 \) and \( p_1 \), where \( p_0 \) is the probability of being attacked (a function of smell) and \( p_1 \) is the probability, for a plant which has just lost one leaf, that it will lose one more leaf immediately (a function of the taste).

The elements of Pj are given by: p(i, k) = 1 − \( p_0 \), if i = k and i ≠ 4 (the probability for a plant not be attacked between day j − 1 and day j); and p(i, k) = 1, if i = k = 4 (when entirely grazed, a plant cannot change). The probability that a plant which has had the first leaves removed (\( p_0 \)) will have i − k − 1 more leaves removed is \( p_1^{i-k-1} \), and that the slug then stops grazing is 1 − \( p_1 \), if there are still leaves on the plant and 1.0 if the plant has lost all its leaves. Thus p(i, k) = \( p_0 \cdot p_1^{i-k-1} \), if k < i and i ≠ 4 and p(i, k) = \( p_0 \cdot p_1^{i-k-1} \), if k < i and i = 4.
The composition of the matrix $\mathbf{P}_j$ is thus:

<table>
<thead>
<tr>
<th>Number of leaves eaten</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>At day $j$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1 - $p_0$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>$p_0(1 - p_1)$</td>
<td>1 - $p_0$</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>$p_0p_1(1 - p_1)$</td>
<td>$p_0(1 - p_1)$</td>
<td>1 - $p_0$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>$p_0p_1^2(1 - p_1)$</td>
<td>$p_0p_1(1 - p_1)$</td>
<td>$p_0(1 - p_1)$</td>
<td>1 - $p_0$</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>$p_0p_1^3$</td>
<td>$p_0p_1^2$</td>
<td>$p_0p_1$</td>
<td>$p_0$</td>
<td>1</td>
</tr>
</tbody>
</table>

The maximum likelihood estimation of parameters $p_0$ and $p_1$ was made using an iterative process (Richards 1961). The final values of the two parameters are those which maximize the probability of obtaining the observed values for $E(j)$.

The estimates were made by the program MLP (Ross 1970; Vila 1977).