Technical note

A simple method of marking plant modules in ecological studies

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SUMMARY

1) A marking method useful in studies on the dynamics of seeds and on the fate and age structure of above-ground modules is described. Seeds and modules were marked with small dots of latex paint.

2) Marking had no effect on the total level of germination of Plantago lanceolata L. However, germination of painted seeds was slightly delayed.

3) Marking of leaves and scapes caused hardly any effect on the growth of Plantago media L. plants in greenhouse experiments.

4) The suitability of the marking method was demonstrated on Plantago major L. in a field experiment.

RÉSUMÉ

1) On décrit une méthode de marquage utile dans les études sur la dynamique des graines et sur le destin et la structure d’âge de modules de plantes. Les graines et les modules sont marquées avec des petits points de peinture ou latex.

2) Ce marquage n’avait pas d’effet sur le niveau total de germination de Plantago lanceolata L. Cependant la germination des graines marquées fut légèrement retardée.

3) Le marquage des feuilles et des hampes n’ont que peu d’effet sur la croissance de Plantago media L. dans des expériences en serres.

4) L’intérêt de cette méthode de marquage a été démontré dans des cultures au champ de Plantago major L.

INTRODUCTION

In studies on plant ecology and, especially in population dynamics many authors have emphasized the need of studying all stages in the life cycle of plants as well as the importance of investigations on the demography of individual plant modules (e.g. Harper, 1977; Solbrig et al., 1979; Blom, 1979). In many cases studies on the fate of seeds and leaves require a marking method that can be used under laboratory and field conditions. A common indirect marking method applied in demographic studies and in sowing experiments in the field is the use of a mapping table; permanent rods in the soil secure the fixed location of the mapping table during the observation period (cf. Mack & Harper, 1977; Mack & Pyke, 1983). A method of direct marking to locate seeds is the use of radio-active tracers. For example, in a study of population dynamics Watkinson (1978) observed the fate of seeds of Vulpia
membranacea L. which were labelled with scandium-46. Willat & Struss (1979) used neutron radiography in the laboratory to investigate the germination and early growth of shoots and roots of Glycine max L., Zea mays L. and Vicia sativa L. Bazzaz & Harper (1977) described a method in which small colour-coded plastic rings were placed on leaves of Linum usitatissimum L. in such a way that different rings rested on leaves of different ages. This method proved to be very useful in experiments under laboratory conditions on vertically growing plants with a relatively simple morphological structure. Under field conditions, however, marks like plastic rings may easily cause damage to stems and leaves. Because of the disadvantages for use in the field of the indirect marking methods mentioned in literature, a direct marking technique was devised, suitable for use under relatively extreme field conditions. Seeds of Plantago lanceolata L. were painted with white Latex wall paint and effects on the germination of marked seeds were studied. This method of marking also appeared applicable in studies in which individual plants are considered a population of modules. As an illustration, the fate and age structure of marked modules of Plantago media L. were studied in a greenhouse experiment. The same was done under relatively extreme conditions in the field for Plantago major.

These experiments were carried out as part of a multidisciplinary project on the relationship between demographic, physiological and genetic properties of some grassland species and the characteristics of their environment. In this project differences in life-history characteristics between and within five Plantago species are being studied (cf. Blom, 1983).

MATERIALS AND METHODS

Sowing experiments

Seeds of Plantago lanceolata from a population in a dry dune grassland (Westduinen, the Netherlands) were used. These seeds were collected in the field and stored at 5° C for two years.

Two series of sowing experiments were performed. Series I was carried out in Petri-dishes with wet filter paper and Series II in pots (capacity 1 litre) filled with dune sand poor in organic matter (less than 2 %). Soil moisture content was 18 % by weight. Before sowing half the number of the seeds was marked with a thin layer of white Latex wall paint so that one half of each seed was covered with paint. In both series 100 marked and 100 unmarked (control) seeds were sown, and the experiments were replicated five times. These experiments were performed in the greenhouse at 18° C and under a photoperiod of 16 hours.

As a comparison 500 marked seeds of Plantago lanceolata were sown in the field (Westduinen) in order to get an impression of the recovery of marked germinated and marked ungerminated seeds after four weeks. A control of 500 unmarked seeds was added. Marked and unmarked seeds were sown in a regular pattern. The ungerminated seeds were recovered by sieving the upper layer (0-5 cm) of the soil.

Marking of above-ground modules of plants growing under greenhouse conditions

Two series of plants (U: unmarked; M: marked) of Plantago media were involved. Each plant was grown in a pot (capacity 3 litres) filled with sand originating from older dunes. Nutrients for optimal growth were added. To analyse the growth of plants the product of the number of leaves and the length of the longest leaf had proved to be a useful non-destructive measure under field conditions (Noë & Blom, 1982). Series U (unmarked plants), serving as a biomass reference series in the laboratory, initially consisted of forty-two plants of which five plants at a time were harvested at regular intervals. The correlation between above-ground biomass and the product of the number of leaves and the length of the longest leaf was 0.78 (P < 0.05). The unmarked plants of series U also served as a reference to series M (marked plants) to detect possible effects of marking on growth.
Series $M$ consisted of fourteen plants; immediately after its appearance, each leaf and each scape was marked with small dots of white paint (Latex wall paint) in such a way that the sequence of appearance could be recognized. Latex wall paint was chosen because no penetration by this paint into the leaf was to be expected. Each week the age of each leaf, the length of the longest leaf, and the number of leaves were determined in both series. Furthermore, each week the length of the longest scape with its spike and the number of spikes per plant were measured, because these characters give an indication of seed production. In the series $M$ all leaves and scapes were marked and additional measurements on each marked module were carried out. These results will be published in a forthcoming paper. The experiments were carried out in the greenhouse during 1982 at a temperature of 22°C and a photoperiod of 16 hours. A similar experiment was carried out with Plantago major ssp. major (see Plate 1).

**Plate 1.** — Each leaf and each scape of Plantago major is marked with small dots of white paint (Latex wall paint) in such a way that the sequence of appearance could be recognized.

*An example of application of the marking method in the field*

To illustrate the applicability of the method in the field, under rigorous conditions, an experiment examining the influence of trampling on plant growth is described. With the method of marking already described the leaf demography of trampled and untrampled individuals of Plantago major ssp. major was studied in experimental plots in the field. The plants were four years old when the measurements described below were started and the trampling treatments had been applied during the whole life time of the plants. The soil of these artificial plots consisted of sand from the older dunes as used in the greenhouse experiments. The experimental field consisted of an untrampled series with a loose soil and a trampled series with a compacted soil. Trampling was simulated by means of a trampling machine (see Blom, 1979). Plants in the trampled series were trampled individually.
six times a day with a force of 0.25 kg/cm\(^{-2}\) which equals the force applied by a man of medium weight (cf. Liddle, 1975). For this experiment ten plants of each series were selected. The leaves of each plant were marked with small dots of white paint (Latex wall paint) in order of appearance. Each fortnight the number of living leaves, and the age of each leaf were determined.

**RESULTS**

*Sowing experiments*

Figure 1 shows that in both series a high percentage of the sown seeds of *Plantago lanceolata* had germinated within 24 days. At the end of the experiment in Petri-dishes significantly more unmarked than marked seeds had germinated \(P < 0.05\), whereas no significant differences in total number of germinated seeds were found in soil. In both series germination of marked seeds was delayed compared with the unmarked ones; in Petri-dishes these differences were forty-eight hours on an average, whereas a difference of twenty-four hours was found in the soil. In the field experiment 89.8\% of the sown marked seeds had been recovered after four weeks. At that time 10.9\% of these recovered seeds had emerged. Hardly any differences in percentage seedling emergence occurred between the marked and unmarked seeds, but not more than 37.4\% of the unmarked seeds had been recovered.

![Fig. 1. — The germination of unmarked seeds of *Plantago lanceolata* (open symbols) as well as of seeds marked with white dots of Latex wall paint (closed symbols). The experiments were carried out in the greenhouse in Petri-dishes (open and closed circles) and in pots filled with a sandy soil (open and closed squares).](image)

**Marking of above-ground modules of plants growing under greenhouse conditions**

Table I shows the mean numbers of leaves and spikes as well as the mean lengths of the longest leaves and scapes with spikes of unmarked and marked *Plantago media* plants. Fortnightly results are given. No important differences in those characteristics were found between both series.

In the other laboratory experiment the differences between the marked and unmarked series of *Plantago major* ssp. *major* were also small. However, at the end of this experiment the leaves and spikes in the marked series became somewhat shorter than those of the unmarked series.
### Table I. — The effect of marking (Latex wall paint) on the development of vegetative and generative modules of individuals of Plantago media.

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of plants</td>
<td>U</td>
<td>M</td>
<td>U</td>
<td>M</td>
<td>U</td>
<td>M</td>
<td>U</td>
<td>M</td>
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<tr>
<td></td>
<td>42</td>
<td>14</td>
<td>42</td>
<td>14</td>
<td>40</td>
<td>14</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td>Number of leaves</td>
<td>(\bar{x})</td>
<td>4.0</td>
<td>3.9</td>
<td>5.4</td>
<td>4.6</td>
<td>8.2</td>
<td>7.1</td>
<td>12.0</td>
</tr>
<tr>
<td>(S. E.)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
<td>0.7</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Length of the longest leaf (mm)</td>
<td>(\bar{x})</td>
<td>8.7</td>
<td>8.5</td>
<td>15.4</td>
<td>12.5</td>
<td>37.9</td>
<td>26.0</td>
<td>71.5</td>
</tr>
<tr>
<td>(S. E.)</td>
<td>0.3</td>
<td>0.5</td>
<td>0.9</td>
<td>1.6</td>
<td>2.5</td>
<td>3.9</td>
<td>3.9</td>
<td>6.3</td>
</tr>
<tr>
<td>Width of the longest leaf (mm)</td>
<td>(\bar{x})</td>
<td>3.9</td>
<td>3.9</td>
<td>9.1</td>
<td>7.7</td>
<td>20.6</td>
<td>14.9</td>
<td>38.8</td>
</tr>
<tr>
<td>(S. E.)</td>
<td>0.2</td>
<td>0.3</td>
<td>0.5</td>
<td>1.1</td>
<td>1.2</td>
<td>2.5</td>
<td>2.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Number of spikes</td>
<td>(\bar{x})</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(S. E.)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Length of the longest spike (mm)</td>
<td>(\bar{x})</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8.1</td>
</tr>
<tr>
<td>(S. E.)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.3</td>
<td>1.6</td>
</tr>
</tbody>
</table>

- U: unmarked plants.
- M: marked plants.
- \(\bar{x}\): mean value.
- \(S. E.\): standard error.

**An example of application of the marking method in the field**

The mean age of the individual leaves of trampled and untrampled plants of *Plantago major* is given in fig. 2. Up to July only slight differences in leaf age between both series were observed; the mean age of the individual leaves was about seven weeks. The leaf age of the untrampled plants did not change during the growing season. In contrast, the trampled plants had many relatively young and short-living leaves (life span about three weeks) in the second half of the season. On an average a total of forty-one leaves was formed on a trampled *Plantago major* plant, whereas in one season twenty-four leaves were found on untrampled plants. Besides differences in leaf age between both series, differences in numbers of living leaves and in the length of the longest leaf were also found (fig. 3a, b). In the greater part of the growing season the mean length of the longest leaf of trampled plants was significantly greater than that of the untrampled plants.

In addition to this, the differences between both series, as far as numbers of flowering individuals and the numbers of spikes per plant are concerned, were striking. Most flowering *Plantago major* plants were found in the trampled series and at the end of August the number of spikes per plant in the trampled series was 6.8 ± 0.6 and 2.0 ± 0.4 (mean ± S. E.) in the untrampled series.
Fig. 2. — The mean age (length of lines) with 95 % confidence limits (•) of successive leaves per Plantago major plant on strongly compacted, heavily trampled soil (left, a) and on loose soil, untrampled (right, b).

**DISCUSSION**

In animal ecology marking methods with paints have been used successfully for a long time (e. g. Southwood, 1976), but in plant ecology examples of non-destructive marking techniques are scarce. Since the properties of a plant population strongly depend on events occurring in the early stages in the life cycle of plants (e. g. Harper, 1977) many authors have reported on theoretical and experimental aspects of these early stages like dispersal, dormancy, seed bank and seedling recruitment (e. g. Levins, 1968; Van der Pijl, 1969; Grime, 1979; Begon & Mortimer, 1981). To follow the fate of individual seeds and seedlings in the field marking can be very useful. One example of such an approach is the work of Watkinson (1978) who used scandium-46 to study the dynamics of seed populations. Seeds marked with radioactive tracers can be recovered easily in the field and no inhibition of germination due to scandium-46 was found (Lawrence & Rediske, 1960), but this method may cause problems in densely populated areas. Mortimer (1974) studied the movement...
of painted seeds on the ground surface and Watkinson (1978) used a small dot of
nail varnish as a visual confirmation of radio-actively-labelled seeds, but the effects
of these paints on germination were not discussed. As was shown in the present paper
painting can be a useful alternative to radio-active labelling because the effects of
the Latex paint on germination are relatively small. Compared with the mapping
techniques of unmarked seeds as described by Mack & Pyke (1983), the painting of
individual seeds provides advantages because horizontal movements of seeds in the
soil can be more easily detected and discrimination of naturally occurring and sown
emerged seeds is possible. It was proved that ungerminated marked seeds could
easily be found after the sieving of the upper soil layers and in the first few days after
the emergence of Plantago lanceolata seedlings, the marked seed coat remained
attached to the cotyledons, which means that recognition of marked seeds after emer­
gence was still possible. The neutron radiography and 14C techniques are commonly
used to study certain aspects of shoot and root growth under laboratory conditions
(e. g. Willatt & Struss, 1979; Noble, 1976; Tietema, 1980). In studies in which
demographic properties of above-ground modules are under investigation, the method
in which parts of plants are marked with paint provides a simpler technique. A pre­
requisite of any marking technique is that it should not affect the longevity or growth.
Although small differences in length of leaves and spikes between marked and unmar­
ked plants were found, it is likely that these differences are rather to be ascribed to
disturbances caused by the higher frequency of touching the plants than to the use of
the small dots of Latex paint; in many cases it was observed that frequent measuring
of plants caused a growth reduction (Van der Toorn & Mook, 1982 and J. P. Van
den Bergh, personal communication). Apparently Plantago media is less susceptible
to this kind of disturbance than Plantago major. The method of marking described
in the present paper was also useful under relatively extreme field conditions as was
shown in the trampling experiment.

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Fig. 3. — Mean number of living leaves (left) and mean length of longest leaf (right) per Plantago
major plant growing in an experimental plot. Crosses: strongly compacted soil, heavily tram­
In conclusion it can be stated that, at least for plants with relatively large seeds, marking with Latex wall paint provides a useful technique for the study of seed dynamics. Furthermore, marking plant modules with small dots of Latex wall paint has a negligible effect on the development of the plant. This simple method had proved to be useful in laboratory and field studies on demographic properties such as leaf age and leaf and spike turnover rates.

ACKNOWLEDGEMENTS

We thank Dr. J. W. Woldendorp and Dr. P. J. M. van der Aart for critically reading the manuscript, Mr. W. J. N. M. Verholt for drawing the figures and Dr. L. A. Boorman for correcting the English text. Grassland Species Research Group. Publ. No. 76.

REFERENCES