BIOASSAYS FOR TESTING THE EFFICACY OF GROUND SPRAYED INSECTICIDES FOR GYPSY MOTH ERADICATION PROGRAMS IN CALIFORNIA\textsuperscript{1}

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Abstract. The insecticides Bacillus thuringiensis (BT), carbaryl, and diflubenzuron were tested in a field-laboratory study for the effect of killing first through third instar gypsy moth larvae on foliage of valley oak trees in California. Larval mortality differed significantly between the BT, the other insecticides and the check treatments. Also, the effects of all three of the insecticides were still apparent 28 days posttreatment.

R\'esum\'e. Les insecticides que sont le Bacillus thuringiensis (Bt), le carbaryl et le diflubenzuron \'etaient test\'es au cours d'une \'etude de laboratoire sur le terrain pour la mortalit\'e primaire des larves de spongivore au troisi\'eme stade inter-mue it ce sur le feuillage de ch\'enes de vall\'ees en Californie. La mortalit\'e larvaire diff\'erait significativement entre le Bt, les deux autres insecticides et le t\'emoin. De plus, les effets des trois insecticides \'etaient encore apparents 28 jours apr\'es le traitement.

The gypsy moth, Lymantria dispar, has been a pest of forests and gardens in North America for over a century (4). Caterpillars of this insect may feed on over 300 species of broad-leaf plants (3, 10, 12) and many species of conifers (11, 13) in western United States. Historically, gypsy moth infestations have been treated with synthetic insecticides (4). Concerns about the effects of synthetic insecticides, such as carbaryl and diflubenzuron, on human health, label restrictions limiting the broadscale use of many pesticides, and costs of eradication programs (2, 6) have prompted investigations into alternative agents for use against the gypsy moth in California.

Bacillus thuringiensis (BT) is a biological control agent that may be an acceptable alternative for gypsy moth eradication (1). BT has been used against gypsy moth infestations in British Columbia, Oregon, and Washington with varying degrees of success regarding pest control/eradication (5, 7). In Oregon, Miller and West (9) noted 100\% mortality in third instars 28 days after treatment of foliage with BT.

Information was needed on the length of time that BT remained active under conditions of weathering on foliage in the climate of central California. This study was designed to evaluate the use of BT as a ground-applied spray. The efficacy of BT was compared with the synthetic insecticides diflubenzuron and carbaryl for the effect of killing early instar gypsy moth larvae on oak foliage.

Methods

In 1985, a 0.5 ha area of 3-5 m tall valley oak trees, Quercus lobata, in Sacramento, California was used as the study site. This site was selected because it represented a California Central Valley oak woodland habitat, the trees were healthy and exhibited vigorous twig growth, the trees were small enough that samples could be obtained at random from any branch, treatment of the entire tree was not difficult, and the trees were well separated from each other so contamination by spray drift would not be a problem. Also, the area was removed from urban-commercial use so we could treat trees that had not been previously treated with insecticides.

Five trees per treatment were sprayed to drip with ground spray equipment using: 1) BT (16 BIU) with 85 gm of Plyac per 379 liters of water, 2) carbaryl at 0.5 kg per 379 liters of water, 3) diflubenzuron at 26.4 g per 379 liters of water, and 4) water. The BT treated trees were sprayed five times at seven day intervals, the carbaryl treated trees were sprayed three times at 14 day intervals and the diflubenzuron treated trees were sprayed twice at 14 day intervals as per label recommendations for each product. Check trees

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were sprayed with water five times at seven day
intervals. Treatments began on April 16, 1985
and were completed on May 14, 1985. Temperature and rainfall were measured from the
time of the first treatment to the collection of the
last leaf sample (Table 1).
Samples consisting of five leaf clusters were
taken at random regarding a north, south, east,
west location. Each tree was sampled at seven
day intervals after the last application of each
treatment. The cut end of each twig was placed in
a sealed water vial (aquaptic). The bags were box-
ed and shipped via overnight express to Corvallis,
Oregon, the nearest quarantine facility studying
the gypsy moth. California laws forbid the
maintenance of gypsy moth cultures within the
state.
The gypsy moth larvae used in the bioassay
were reared from egg masses collected in Oregon
during the previous winter. Five gypsy moth larvae
of the same instar (first through third instar larvae
were used) were placed in a 0.5 liter container
with the foliage of each test tree (5 replicates; n =
25/instar/treatment/date). The first instars were
less than eight hours old since egg hatch and had
not previously fed. The second and third instars
were less than eight hours old since molting and
as newly molted larvae they had not fed; however,
in the preceeding instars they had been reared on
an artificial diet (Bioserve #953L1, gypsy moth
diet). Larvae previously fed artificial diet readily
accepted the oak foliage. Mortality of larvae in the
bioassays was evaluated at eight hour intervals for
days 1-4 post-treatment and at 12 hour intervals
thereafter.

Results and Discussion
Because mortality between instars was not
significantly different, the data were pooled for all
larvae. Mortality was highest in the carbaryl and
diflubenzuron treatments, all larvae died in each
test (Table 2). However, larval mortality on the BT
treated foliage did remain high (greater than 80%)
for at least four weeks. Although these data indi-
cated that BT was statistically less effective
compared to diflubenzuron and carbaryl against
the gypsy moth, the testing methods, biological
ramifications, and field application procedures for
each treatment need to be considered in the deci-
sion on which treatment may be used.
Typically, field-laboratory bioassays are open to
critique according to methodology. The results
from this test were a product of treating foliage in
the field in California where the treatments
weathered in local natural conditions (Table 1),
but involved the bagging and shipping of foliage
samples to Oregon. The methods were logistically
awkward but their influence on the data were
unknown.
The results of this study are consistent with the
findings of Miller and West (9) where samples
were taken from the field after weathering under
local natural conditions and assayed immediately
in the laboratory. Miller and West (9) observed
100% larval mortality up to 28 days following
treatment of foliage with BT. The BT was tested
against third instar gypsy moth larvae on foliage of
Douglas-fir, *Pseudotsuga menziesii* and Oregon
white oak, *Quercus garryana*. Thus, in very dif-
ferent habitats, different host plants, and climates,
BT treatments were demonstrated to be effective
well past the 5-10 day treatment interval currently

### Table 1. Weather conditions (temperature, °C; precipitation, mm) during the study period for the bioassay of Bacillus thuringiensis, carbaryl, and diflubenzuron, for larval mortality of the gypsy moth. Sacramento, California, 1985.

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature High</th>
<th>Temperature Low</th>
<th>Precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 16-22</td>
<td>16-24</td>
<td>7-9</td>
<td>2.5</td>
</tr>
<tr>
<td>April 23-30</td>
<td>22-32</td>
<td>7-12</td>
<td>0</td>
</tr>
<tr>
<td>May 1-7</td>
<td>25-32</td>
<td>10-13</td>
<td>0</td>
</tr>
<tr>
<td>May 8-14</td>
<td>21-31</td>
<td>6-11</td>
<td>0</td>
</tr>
<tr>
<td>May 15-21</td>
<td>21-33</td>
<td>8-11</td>
<td>0</td>
</tr>
<tr>
<td>May 22-28</td>
<td>18-33</td>
<td>9-14</td>
<td>0.5</td>
</tr>
<tr>
<td>May 29-June 4</td>
<td>20-32</td>
<td>7-14</td>
<td>3.5</td>
</tr>
<tr>
<td>June 5-11</td>
<td>31-37</td>
<td>14-19</td>
<td>0</td>
</tr>
</tbody>
</table>

1. Range of mean daily temperatures.

### Table 2. Percent mortality of gypsy moth larvae fed leaves of *Quercus lobata* treated with Bacillus thuringiensis (BT), diflubenzuron, or carbaryl, Sacramento, CA, 1985.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Check</th>
<th>BT</th>
<th>Carbaryl</th>
<th>Diflubenzuron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days Post-Treatment</td>
<td>7</td>
<td>14</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>Check</td>
<td>0a</td>
<td>28a</td>
<td>9a</td>
<td>34a</td>
</tr>
<tr>
<td>BT</td>
<td>81b</td>
<td>86b</td>
<td>94b</td>
<td>92b</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>100c</td>
<td>100c</td>
<td>100c</td>
<td>100c</td>
</tr>
<tr>
<td>Diflubenzuron</td>
<td>100c</td>
<td>100c</td>
<td>100c</td>
<td>100c</td>
</tr>
</tbody>
</table>

1. values followed by different letters differ at P < 0.05, ANOVA: data changed to arcsine prior to analysis.
prescribed in the California eradication programs.

Generally, the two synthetic insecticides would not be applied to the point of drip in a larger scale control or eradication program. On the other hand, BT not only could be used in high doses in a large scale program involving livestock, dwellings, and streams, but it could be applied at intervals shorter than the 28 day period of effectiveness we observed. The effectiveness and the safety regarding nontarget, human health issues of BT suggests it could be a ground spray substitute for synthetic insecticides in gypsy moth control programs. If 90% mortality was observed after each application of BT, then two or three properly times applications could approach 98-99% mortality in a field population. This extrapolation for control efficacy would most likely occur with a ground spray to drip or the use of high doses, 16-18 BIU, applied aerially at 14-21 day intervals. In fact, the gypsy moth has declined in Oregon during such an intensive use of BT (16-18 BIU, applied at 7-10 day intervals, three times in May) in a three year eradication program (7). However, recent studies indicate that management decisions should consider how to apply BT in order to alleviate negative impacts on nontarget organisms occurring in the environment being sprayed (8).

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Literature Cited