CONSIDERATIONS IN REARING BRADYRHOA GILVEOLELLA FOR
CONTROL OF CHONDRILLA JUNCEA IN AUSTRALIA

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ABSTRACT

Larvae of the moth Bradyrhoa gilveolella (Tr.) (Lepidoptera:Phycitidae), first imported in 1973, feed
on the roots of skeleton weed, but rearing was only possible on an artificial diet at above average
temperatures. Field establishment of this initial colony proved impossible and first instar larvae had appar-
etly become poorly adapted to fresh plant material and field temperatures during laboratory rearing. A
second colony was imported in 1977, carefully reared and major releases made in 1978/79. Recoveries have
been made and establishment seems likely.

INTRODUCTION

There are several documented examples of changes in the properties of insects produced during maintenance of laboratories colonies (Guthrie and Carter 1972, Haskell et al. 1962, Kajita 1973) some of which have led to the production of laboratory ecotypes no longer well adapted to field survival (House 1967). Few of these are from classical biological control programs, though it is often suspected that inadvertent selection during rearing may have a considerable influence on the outcome of such programs. Frick and Johnson's (1972) account of the decrease from 17 to 6 weeks in the egg diapause of a Swiss strain of Longitarsus jacobaeae Waterhouse (Coleoptera:Chrysomelidae) during eight generations of laboratory rearing is one example from biological control of weeds work. This population was not considered worth releasing.

It is perhaps surprising that with the quantity of insects reared for biological control, there are not more cases known. I believe that this is often because the information is not available to determine whether selection has occurred and also because the means of avoiding or correcting laboratory selection may be unknown or unavailable. The following account is concerned with several points which have emerged from a rather long and complex study of the rearing and colonization of the moth Bradyrhoa gilveolella (Treitschke) (Lepidoptera: Phycitidae). Laboratory selection was almost certainly a significant factor in the fate of attempts at establishing this species in the field.

HISTORY OF THE FIRST INTRODUCTION

B. gilveolella was first introduced as a possible biological control agent for skeleton weed, Chondrilla juncea L. (Compositae) in 1973. Its biology was described by Caresche and Wapshere (1975). Larvae feed on the root of the plant, cutting deep furrows around and sometimes through the root, and enclosing themselves in a tunnel of frass, soil particles, silk and latex. Pupae are formed a few centimetres below the soil surface in exit tubes constructed by the larvae. Adults are free flying, and oviposit around the bases of the plants. The newly hatched larvae feed initially around the bases of the leaves deep inside rosettes until descending to the root. There are two to three generations per year in Greece, from where the first population was obtained.

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When the population was received in Canberra as 602 pupae in 1973, it had not been bred successfully in the laboratory for a complete generation and a considerable amount of work was carried out to determine a successful rearing method. It proved comparatively easy to obtain mating and oviposition in conveniently sized cages, but the maintenance of larvae for complete development was extremely difficult. Production of adults from larvae kept on potted plants was very poor and unpredictable (e.g., five adults were obtained from 1200 larvae on 300 plants in one trial) while maintaining cut root pieces in a variety of containers was not very much better and extremely labour intensive (principally due to continual breakdown of the root material over a very long larval development period). It was finally necessary to develop an artificial diet for the larvae (Cullen, unpub.). At this critical period there was a severe bottleneck in production and from an original emergence of 177 females from the imported material, subsequent generations were derived from the progeny of between three and nine females. (It was not possible to be more exact, as single pair matings were not always used and due to prolonged development times, crosses between first and second generation adults occurred.)

During trials with the artificial diet, first instar larvae established much more readily if they were active, and therefore higher temperatures were used. It was also found that at normal laboratory temperatures (23 ± 2°C) larval development times were very prolonged (three to five months) and extremely variable, with adult emergence from larvae hatching the same day being spread over three to four months. Higher temperatures reduced the larval period to a more manageable length and very greatly reduced the spread of emergence. Thus a breeding colony was established using an artificial diet, with all larval establishment and development occurring at 29°C.

Numbers were gradually increased in this colony, but only a limited release was possible during the 1974/75 season due to other technical problems (Cullen, unpub.), and these insects did not become established. However in the 1975/76 season, a major attempt at establishment was possible. A total of nearly 22,000 first instar larvae, 639 mature larvae and 2000 adults were released at one site. First instar larvae were the first choice for release as they were readily available in quantity and their habit of moving rapidly into the centre of the rosettes seemed to protect them from predation. Five hundred of the adults were released in a large walk-in cage (3.66 m x 1.8 m x 1.8 m high), the remainder were released free in the same area. Adults were observed around the release site up to 10 days after release, and mating and oviposition were observed in the cage.

At various times during and immediately following the release period, samples of plants colonized by the first instar larvae and plants in the large cage were examined. Not a single larva or sign of establishment was observed. On the plants colonized with mature larvae, several tunnels and a few pupae and exuviae were found. However, later samples of plants and trapping for adults using virgin female baited pheromone traps, did not show any signs of subsequent progeny. Similar sampling and trapping in the next season also failed to reveal any such signs and it was concluded that the insects did not establish.

All indications were that first instar larvae had failed to establish, whether colonized directly, or resulting from eggs laid in the cage or by any free flying adults. A simple experiment was carried out to test this hypothesis.
MATERIALS AND METHODS

Newly hatched, first instar larvae of *B. gilveolella* were individually placed on potted plants of *C. juncea* or on artificial diet held at a constant 22 or 29°C or in alternating regimes of 16:26°C (12:12 h) or 8:18°C (12:12 h). The photophase was 12 h except at constant 29°C where it was 14 h. All were examined between two and three weeks later. Replicates of 50 larvae were used for each treatment but unfortunately only two replicates were conducted before the whole colony decreased further in vigour and became difficult to maintain.

RESULTS

Decreased ability of the larvae to establish on fresh plant material and at lower temperatures are tentative conclusions from this experiment (Table 1).

Table 1. Percentage establishment of first instar larvae on artificial diet and on plants.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Diet</th>
<th>Plants</th>
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<tbody>
<tr>
<td>29 constant</td>
<td>86.0</td>
<td>15.0</td>
</tr>
<tr>
<td>22 constant</td>
<td>73.3</td>
<td>6.0</td>
</tr>
<tr>
<td>26/16 alternating</td>
<td>67.0</td>
<td>15.0</td>
</tr>
<tr>
<td>18/8 alternating</td>
<td>26.0</td>
<td>16.0</td>
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</table>

The females which emerged from the imported pupae laid an average of 130.6 eggs each (*n* = 64). This declined to 89.9 (*n* = 45) and 77.5 (*n* = 616) for two generations around the time of release, with a fertility only slightly in excess of the 19.2 per cent hatchability recorded in the second. Fecundity declined further during 1976 and the colony became extremely difficult to maintain. This, with the results above, suggested that further work with this colony was unjustified.

THE SECOND INTRODUCTION

In October 1977 a new population of *B. gilveolella* consisting of 207 larvae was imported from the same areas of Greece. The larvae were removed from their burrows and placed on artificial diet at a temperature of 22 ± 2°C. Once they were observed tunnelling and feeding, the smaller and more slowly developing larvae were transferred to 29°C to better synchronize emergence of the small number of adults expected. A colony was successfully established with a founding population of between 19 and 29 females. A total of 39 females and 35 males were in fact obtained from the 207 larvae. (Ten females were definitely known not to have contributed to the colony.) Approximately 77 per cent of the larvae which failed to produce adults were parasitized, succumbed to disease, or were injured during their removal from their burrows. Some larvae did not accept the artificial diet immediately but were encouraged to start feeding by providing them with pieces of root. Once feeding commenced, they would then usually move to the medium. A few larvae failed to establish on either and died of starvation, while some which fed on the medium developed poorly and eventually died. There were only 14 of the latter however, and it seemed that selection for ability to develop on the artificial diet was not restricting variability significantly.
Other precautions were taken with this colony. First instar larvae were always established on the diet at 19 to 22°C. In contrast with the first population, these larvae appeared more vigorous and establishment rates were high at this temperature (80 to 90 per cent). After seven days, larvae were transferred to 29°C as it was still necessary to shorten development times and better synchronize emergence.

Initially, all emerging adults were used to increase the colony, but after two generations there was a surplus and some selection was necessary. The adults chosen to continue the colony included representatives from all batches of larvae and also individuals which had developed at different rates. The remainder of the adults were culled (or released). All eggs obtained were counted and allowed to hatch and regular fertility checks made. Except initially and during release, there was a surplus of hatching larvae and again a selection was made, this time to include different parents, dates of oviposition and times to hatching.

Under this system, a generation took approximately 11 weeks. After the initial increase and unless producing large numbers for release, the colony was established at a level of 25 females used for rearing each week and 200 larvae established on diet. Given a relative shortage of one essential diet ingredient and a scarcity of labour, this was considered the minimum for maintaining a healthy colony. As the rearing was continuous, this represents 275 females per generation being selected from the adults reared from 2 200 larvae. There was thus no major mortality at any stage other than that imposed and controlled by ourselves.

FIELD RELEASES

From September 1978, larvae and adults of *B. gilveolella* were released at two sites near Canberra during spring and summer 1978/79. At one site, 1624 first instar larvae, 4004 mature larvae and 930 uncaged adults were released; and 6629 first instar larvae, 1691 mature larvae and 1832 uncaged adults were released at the second site. The first larvae released were the third generation since importation. At this time fecundity was 116.6 eggs per female (*n* = 105) with a fertility of 37.8 per cent.

Some evidence of larval establishment was obtained from samples of plants from the release sites but further disturbance of the sites was not considered advisable. However, some of the results of an experiment carried out at the same time to investigate release and establishment techniques permit an estimate of likely field establishment. As part of this wider investigation, newly hatched first instar larvae were colonized on potted plants of two different ages, which were then kept either in the field, or in laboratory conditions at a temperature of 23 ± 2°C. The plants were well grown, but the younger ones (six months old approx.) were still succulent while the second group had been kept outside for at least 12 months in addition, and had older, tougher crowns with the remains of old leaves, stems, etc. These were very similar to the condition of plants in the field while the younger ones were similar to those used in the experiment carried out with the first colony.

RESULTS

Percentage establishment of first instar larvae on young and old plants under field and laboratory conditions is summarized in Table 2.
Table 2. Percentage establishment of first instar larvae on plants.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Young plants</th>
<th>Old plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second population:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>68.0</td>
<td>28.3</td>
</tr>
<tr>
<td>Field</td>
<td>23.3</td>
<td>10.0</td>
</tr>
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</table>

This experiment was not designed for comparison with that performed previously with the first population and the results are not strictly comparable. However, the plant quality and rearing conditions were very similar for the 22°C treatment with the first population (Table 1) and the 23°C treatment with the second population (Table 2). The results suggest that the first instar larvae of the second population were better able to establish on plants than those of the first population, and that establishment on older plants under field conditions was much less than on younger plants under protected conditions. Combined with the nil recovery on plants in the field following the massive effort made in 1975/76, the indications are that failure to establish on plants in the field was an important characteristic of the first population and that this was avoided with the second introduction.

In the 1979/80 season, recoveries were made of a few adults at both sites. In one case this was after at least two generations in the field since the last releases; this was probably also true in the second case. Numbers were very small, but establishment seems likely at both sites.

**DISCUSSION**

In considering this case history (with the advantage of hindsight), it is instructive to consider what improvements could have been made both in monitoring fitness of the populations and also in rearing techniques. It seems desirable to monitor a few common parameters, whatever the organism involved. The exact size of the founder population (i.e., females actually contributing to the laboratory colony), can be important as will be the size of the effective breeding population through subsequent generations. Fecundity and fertility of individual females could also be useful as a check on overall vigour and possibly of increasing homozygosity in the population. Some of this information was gathered in this case, but to monitor these factors completely would have required isolation of females and single pair matings, or post mortem determination of reproductive status. The latter was attempted, but could not be developed to a reliable technique. Data collected early with the first colony showed that the chances of obtaining successful mating and fertile eggs was significantly reduced when single pairs were kept alone (Cullen, unpub.), so this was not considered desirable, when the prime aim was to increase the number of females contributing to the population.

It is possible that isozyme analysis might provide a technique for monitoring variability and change in a laboratory colony. This was not attempted in this case though the technique is being developed for use in other cultures. Perhaps another general recommendation arising from this example is that if gross departures from normal experience are necessary (e.g., the use of artificial diets), checks should be incorporated to occasionally test the suitability of the original
natural conditions, i.e., in this case the natural food plant. This will be done in future.

It would clearly also be desirable to monitor closely those properties of a population which affect later success in the field. This is a more difficult proposition. The insect's fitness, its ability to establish and its ability to damage the plant are all closely interrelated but not necessarily the same and each involves several properties which may not be at all obvious. In this particular case, it seems that establishment of first instar larvae was a critical point, but this was not clear at the beginning, when certain vital decisions had to be made. This is a general problem at the beginning of a rearing program with a new insect. This, plus the technical difficulties involved in actually establishing a culture, can make it impossible to establish any standards for later comparison before significant changes take place. When numbers are very low there are not insects to spare for experiments which involve significant mortality. This particular case also only considered one property. What others might have been affected is unknown.

In this state of ignorance, what perhaps could have been done about rearing techniques which might have retarded any genetic decay or adverse selection? Boller (1972) made several suggestions about maintaining natural behaviour in a laboratory colony, the first of which was to define the problem and the most important properties of the insect along with appropriate tests, but the difficulty of achieving this has already been discussed. Boller (1972) also recommended maintaining populations at high yield, not selecting too early for standardization and the incorporation of "luxury features". High yields are obviously acceptable to a biological control worker, if attainable. It is often during the initial process of trying to find a method of obtaining those high yields that problems can occur. Later, as in this case, mortality can be kept under the control of the research worker. While biological control workers would in general not aim for a standardized insect, the concept is relevant in that it is common practice to attempt to standardize a rearing procedure which can then be followed by a technician. It is sometimes impractical to do otherwise and again, there are often good arguments for switching one's rearing systems to the first one which seems to work reliably, in order to obtain as many individuals as possible, particularly during the initial stages. However, perhaps we should give more thought to varying rearing conditions fairly frequently, even to having several standard systems available which could be alternated, though this may only be possible in later stages of rearing. To some extent, the incorporation of different characteristics (which are not essential or even sometimes slightly deleterious to a maximum rate of production) into such systems constitutes the inclusion of 'luxury features' as suggested by Boller (1972). The over-riding consideration will always be whether it is possible, for example, to have delays in the system by using lower or field temperatures, or to have large cages for mating, fluctuating temperatures for rearing, etc. There are often severe technical difficulties. Two groups of larvae of *B. gilveolella* were reared in an alternating temperature regime of 30/20°C but development and emergence were so prolonged and uneven that it was impractical for normal rearing.

Performing tests on insect colonies is difficult if it is not known what factors are important and there are no standards with which to compare the results. However, when there is some indication of possibly important features, it is obviously desirable to try and confirm their importance and if possible, improve
the technique. The use of natural conditions, as suggested by Boller (1972) as
a cure, is again a matter of practicality, particularly if material has to be main-
tained in quarantine and technical facilities are not extensive. Crossing with wild
populations is obviously usually impossible, but perhaps some consideration
should be given to trying to maintain two or more populations, one at least of
which might be maintained in as close to natural conditions as possible, even if
yields are not high and development times are prolonged. This might provide a
source of less laboratory-selected individuals for improving the quality of a
mass produced population at a later time.

The ultimate test is of course performance in the field and I strongly suggest
that follow up work should be adequate to determine whether there is a problem
which is traceable to the rearing techniques employed.

What can one conclude from this perennial conflict of necessity and desirabil-
ity? Perhaps the most that can be expected is that if we are all aware of the
type of problems which might arise and the need to monitor them, we might
be able to avoid the worst ones and at the same time gain some insight which
might be of use in future work.

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