SEED AND STEM WEEVILS OF PUNCTUREVINE: A COMPARATIVE STUDY OF IMPACT, INTERACTION, AND INSECT STRATEGY

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ABSTRACT

In a field experiment, the effects of seed and stem weevils on stem length, flower production, growth rate, water stress, biomass, and seed germination of puncturevine (\textit{Tribulus terrestris} L.) were compared to the same plant damage parameters of plants in an insecticide check. Stem weevils caused the greater impact, significantly affecting all parameters by destroying vascular tissues. Stem weevil larvae caused extensive damage to pith, xylem, phloem, and woody tissues. Seed weevils caused significant increases in flower production, thus providing an increase in the number of oviposition sites, which suggests survival strategy; however, seed weevils caused drastic reductions in per cent seed germination.

INTRODUCTION

The primary purpose of this research was to evaluate the impact of two imported biological control agents on the noxious plant puncturevine (\textit{Tribulus terrestris} L.). This weedy caltrop, believed to be native to North Africa and the Mediterranean region, was first reported in the United States (California) by Davidson (1903). It has since spread to most of the northern states and has become especially noxious in dry, sandy soils in the West and Southwest (Reed and Hughes 1970).

The puncturevine seed weevil, \textit{Microlarimus lareynii} (Jacquelin du Val), and the puncturevine stem weevil, \textit{M. lypriformis} (Wollaston), were introduced for control of puncturevine in the United States in 1961 after studies of their ecology and host plant specificity were conducted in Italy by Andres and Angalet (1963). The progress of these weevils was initially reported by Huffaker \textit{et al.} (1961); their colonization and spread in the United States was reported by Maddox (1976); and their current status in California was reported by Maddox and Andres (1979).

Prior to this study, no attempt had been made to assess the impact of these weevil species on their host plant, although Kirkland and Goeden (1978b) assessed the impact of populations composed of both seed and stem weevils on puncturevine plants field-grown under irrigated and nonirrigated conditions. (They compared differences in plant mortality, flower and seed production, and stem lengths [radii] between insecticide-treated and untreated plants.)

This field study was designed to assess the impact of seed and stem weevil species independently upon their host plant, puncturevine, by use of physiological and other parameters.

METHODS AND MATERIALS

Establishment of field groups

Three rectangular groups of 15 plants each were established in the 'Armstrong field area' of the Department of Plant Pathology at the University of California at Davis, CA, with a seed weevil infested group, a control group, and a stem

\footnote{1 Biological Control of Weeds Laboratory, AR, SEA, USDA, 1050 San Pablo Avenue, Albany, CA 94706, U.S.A.}
weevil-infested group. The distance between each group was 11 m, and the control group was planted between the seed and stem weevil groups as an additional buffer zone. The 7.3 x 11-m seed weevil group contained three rows of five plants/row. The 3.7 x 12.3-m control and stem weevil groups contained one row of seven and one row of eight plants each. In the seed weevil group, rows were 2.4 m apart, and plants within a row were 2.7 m apart; in the other groups, rows were 1.8 m apart, and plants within a row were 1.5 m apart (Figure 1). The control group was sprayed with Isotox2 initially and then once each week to serve as an 'insecticidal check' for comparison with the 'weevil-infested' groups.

Plant culture, standardization

Puncturevine plants were grown to about 12 cm in diameter in the laboratory from seed that was field-collected the previous year at Manteca, CA. Plants of equal size were transplanted in the respective groups on 13 June 1979. All plants were treated with Rootone F2 with fungicide to prevent damping-off, shock, and to promote growth, and were watered once per week for two weeks. No water was added thereafter. A 1.2 x 1.2-m wire mesh screen placed under each plant captured about 95 per cent of the dehiscing carpels. A central hole and a lateral cut were made in each screen to facilitate their placement under the plants. The corners of each screen were anchored with iron rods.

Weevil collection, plant infestation

All adult weevils initially collected from field populations were separated according to species. Infestation dates and number of adult weevils used were as follows. Seed weevil group: 16 July 1979, 6/plant; 23 July, 5/plant; 30 July, 6/plant; 14 August, 8/plant. Stem weevil group: 16 July 1979, 5/plant; 23 July, 4/plant; 30 July, 2/plant. Thus, a total of 25 seed weevils and 11 stem weevils were used per plant in their respective groups.

Stem internode length

Selection was made during sampling from the centre crown in a clockwise direction to avoid any bias regarding stem length. The number of stem internodes in each 30-cm length of stem (measured from stem tip) on each of the four tagged stems of each plant in the three groups was counted weekly and the average number and average length of internodes determined.

Floral counts

Flower counts were made as a measure of flower production. Each plant was divided into quadrants with two perpendicular rods, and flowers were counted in each quadrant with a hand-held mechanical counter. Flowers were counted each week on each plant in each group. Groups were counted in succession before any other parameters were examined so as to keep the groups comparable. Because the flowers are ephemeral and open widely on warm, sunny mornings (Squires 1969), they were counted during the morning.

Adult weevil feeding

Feeding scars of adult weevils on all plants were counted weekly on 10 cm of

2 This paper reports the results of research only. Mention of a pesticide in this paper does not constitute a recommendation for use by the USDA nor does it imply registration under FIFRA as amended. Also, mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the USDA.
each tagged stem (measured from stem tip).

**Plant growth rate**

On 22 August, the tagged stems on each plant were marked 10 cm in from the tip with a red pen. On 29 August and on 5 and 11 September, each tagged stem was again marked 10 cm from the stem tip, then the distance between that and the mark from the preceding week was measured. Sometimes only two or three stems on a plant were measured, although each was marked.

**Plant water stress measurements**

The water stress of the plants in each group was measured with a Model 3005 'Plant water stress console'² (pressure chamber) (Figure 2) to determine the impact of weevil feeding on plant water stress. Both nocturnal and diurnal measurements were recorded, representing low and high metabolic levels, respectively. Nocturnal measurements were recorded on 14 September from 0205 to 0623 hr, and diurnal measurements were recorded on 17 September from 1105 to 1439 hr. To make the groups comparable in time, the same replicate number in each group was measured before we proceeded to the next replicate number. The average nocturnal and diurnal temperatures and RH recorded during the period of the measurements were 13°C, 87 per cent and 30°C, 45 per cent, respectively.

The measuring procedure was as follows. An apical growing stem (6 cm long) was randomly selected on the plant, excised, and inserted into the pressure chamber. When the equilibrium pressure for the sample was reached, the sap commenced to flow from the exposed cut end of the sample, and the pressure reading was recorded. This value was equivalent to the negative force with which the plant water was held within that sample, referred to as 'plant water potential' or 'plant water stress'. Readings were replicated twice for each plant.

**Histological assessment of weevil feeding**

Field-collected puncturevine stem material (ca. 4 cm long) was placed in formalin-acetic acid-ethanol (FAA) and allowed to remain until just prior to sectioning. The material was then washed with dist. H₂O several times and placed under a vacuum for removal of the FAA.

Sectioning was performed with a freeze microtome. The sections were cut into ribbons (14μ/section) and mounted according to standard microtome procedure, then examined and photographed for determination of the plant tissues fed upon by each weevil species.

**Plant biomass determination**

Plants from both 'weevil' and 'control' groups were harvested on 19 and 26 September 1979. Individual plants were excised at ground level (excluding roots) and deposited in paper bags, then the bags were sealed and dried at 49°C until a stabilized dry weight was obtained.

**Determining seed weevil impact on germination**

Seeds from the seed weevil and control groups were randomly collected on 10 October and sorted into two lots, 'fed upon seeds' and 'not fed upon' seeds. The separate lots were again randomized for use in the germination test.

The germination test was conducted in a 'Stultz Daylight Germinator'. Seeds were germinated at 16 hr light at 20°C, 8 hr darkness at 35°C, and RH nearly 100 per cent. The seed sample consisted of 100 carpels/replicate, four replicates of each treatment. Randomly selected carpels were placed between moist, folded
PLANT WATER STRESS CONSOLE
A - Specimen holder  E - Pressure control valve
B - Pressure chamber  F - Sample board
C - Pressure test gauge  G - Nitrogen supply tank
D - Metering valve  H - Chassis

Figure 2. Photo of Model 3005, 'Plant water status console' used to measure plant water stress.
blotters for five days. After five days, the carpels were scraped, and those that had germinated were counted and removed. The test was then continued; a second count was made after seven days and a third after 19 days.

**Weevil interaction**

To monitor the movement of adult seed weevils, a sample of 60 seed pods/group was collected from all groups, with four seed pods collected randomly from each plant. These were dissected and the per cent infestation determined for each group. (The seed weevil and stem weevil groups were a minimum of 34.3 m and an average of 46 m apart.)

The movement of adult stem weevils was estimated by counting the number of emergence holes in four stems/plant in all groups. A standard 50 cm/stem length was examined for weevil emergence holes.

**Statistical analysis**

The method chosen for data summarization was 95 per cent confidence intervals on treatment group means. For repeated measurements across time, analyses were performed separately by dates. Although the philosophy of the confidence interval approach is different from that of significance testing, non-overlapping confidence intervals infer significance. (The actual significance level in this case is less than 1 per cent.) Within plant data was either averaged or totalled before analyses both for simplification and because sub-samples were not needed for estimation of the error terms. Transformation was determined according to a method given in Box *et al.* (1978).

**RESULTS AND DISCUSSION**

**Stem internode length**

A comparison of the group means across time showed that the average number of internodes per 30-cm stem length increased significantly in both seed and stem weevil groups versus the control group, an indication of a reduction in the internodal length of the stems. By 11 September, the seed and stem weevil groups averaged 3.1 and 3.8/stems per plant more internodes, respectively, than the control group. The 95 per cent confidence intervals inferred significance (actual significance in this case was less than 1 per cent) (Figure 3).

These results indicate that both weevil species cause significant shortening in the length of the stem internode of *T. terrestris* and that the stem weevil causes the greater change in this parameter. Physiological stress is most likely induced by stem larval feeding, which affects vascular tissues and thus interrupts the transport mechanism of the plant. Seed weevil feeding, by comparison, does not affect the vascular tissues, but is believed instead to divert plant growth substances to the feeding sites on the epidermis of the stem (adult feeding) or in the developing carpels (larval feeding), hence reducing the availability of these growth substances for stem elongation. A diversion of growth substances to feeding sites may result as part of the phenomena of wound healing in plants. 

Esau (1960) pointed out that both secondary growth and cambial activity are often involved in wound healing phenomena, and injuries that occur on plant parts not having secondary growth usually result in the formation of a cicatrice. Moreover, larval feeding in the carpels may induce nutrient sinks, thus diverting growth substances. These may be similar to the ‘limited neoplasms’ discussed by Mani (1964), who described the non-biological limited neoplasms as galls induced by either physical or chemical means.
Figure 3. Ninety-five per cent confidence intervals on treatment group means of number of stem internodes per 30 cm of stem for repeated measures across time by date.

Floral counts

Plants in the seed weevil group produced significantly more flowers than those in either the control or stem weevil groups, and plants in the stem weevil group produced significantly fewer flowers than those in either the control or seed weevil groups (Figure 4).

The number of flowers in the seed weevil group increased significantly with time. The specific mode of action stimulating an increase in the number of flowers and subsequent carpel production in *T. terrestris* is not known. However, heavy pruning has been shown to occasionally stimulate flowering in plants. Furthermore, other processes that interfere with vegetative growth also stimulate flower development. Growth inhibition in stems causes stunting that is sometimes accompanied by increased flowering as indicated by a greater number of flowers per plant (Salisbury and Ross [p.647] 1969). Also, shortening of the stem internode in this experiment resulted in production of more stem nodes, with the flowers occurring at these axillary sites.
Figure 4. Ninety-five per cent confidence intervals on means square roots-number of flowers for repeated measures across time by date.

Independently, the effect of seed and stem weevil species on flower production in *T. terrestris* is quite different, as can be seen in Figure 4. However, in mixed populations, flower production is significantly reduced, as reported by Kirkland and Goeden (1978a) and in an unpublished study of Maddox et al. My studies suggest that, in a mixed population of weevils, the impact of the stem weevil on its host is greater than that of the seed weevil because it affects critical vascular tissues.

The increased carpel production greatly increased the number of available oviposition sites for the female seed weevil, and thus would enhance the density and genetic composition of the seed weevil population. Since the feeding effect of the seed weevil on *T. terrestris* resulted in a demonstrable increase in the numbers of flowers and subsequent carpel production, oviposition sites, and population density, these events suggest a possible use of a survival strategy by the
seed weevil to maximize its host plant as a food resource for its own development and survival.

Adult weevil feeding
The square root of the mean number of feeding scars/plant was significantly different between groups across time. Adult stem weevils fed more upon *T. terrestris* than the seed weevil (Figure 5).

![Graph](image)

*Figure 5. Ninety-five per cent confidence intervals on means square roots-number of feeding scars for repeated measures across time by date.*

Although the initial ratio of adult weevils used to infest the plant groups was 2.1:1 seed weevil:stem weevil, this ratio changed with time to as high as about 10:1. In spite of this change, the data suggest that individual adult stem weevils fed more than individual adult seed weevils.
Rate of growth in plant groups

Plants in both seed and stem weevil groups grew significantly less than those in the control group, and plants in the stem weevil group grew significantly less than those in the seed weevil group (Figure 6).

Figure 6. Ninety-five per cent confidence intervals on log average growth for repeated measures across time.

These differences in rates of growth suggest that the degree of physiological stress measured in *T. terrestris* is very much dependent upon the kind of tissue(s) affected by weevil feeding, also reflected in the other parameters measured. Essentially, stress appears to be more severe when vascular tissues are involved, such as in stem weevil larval feeding.

Plant water stress measurements

Measurements of plant water stress during the nocturnal cycle showed significant differences between groups. Plants in the stem weevil group were
stressed most, with an average of 5.9 bars of pressure required to initiate sap flow. The seed weevil group required an average of 5.1 bars, whereas the control group required only 3.7 bars to initiate sap flow (Figure 7).

Measurements of plant water stress during the diurnal cycle showed significant differences between the weevil groups and the control, but not between the weevil groups themselves. Water stress in plants in the stem weevil group averaged 16.1 bars of pressure versus 16.0 bars for the seed weevil group and only 13.5 bars for the control group (Figure 7).

Measurement of moisture stress as an indicative parameter of change in stressed plants as a result of insect feeding was first reported by Ferrell (1974) in studies of the attack for fir engravers, *Scolytus ventralis* LeConte, in white fir, *Abies concolor* (Gord. and Glend.) Lindl. ex Hildebr., infected by true mistletoe. Ferrell (1978) also studied the moisture stress threshold of susceptibility in white fir to fir engraver beetles with a pressure chamber technique to identify white firs that were resistant or susceptible to beetle attack. In 1979, R.W. Pemberton called my attention to this technique, and Pemberton and Andres (1980) reported the feeding impact of *Coleophrora partitibica* Meyrick on *Salsola australis* R. Br. with the pressure bomb technique.

**Histological assessment of weevil feeding**

A comparison of the histology of stem cross sections of *T. terrestris* for differences in feeding damage caused by adult seed and stem weevils showed that the adults of both species of weevils feed on external tissue, not including vascular tissue. Tissues consumed and damaged, for the most part, consist of the epidermis, cortex, and fibre bundles located within the cortex (Figure 8). Surface desiccation of underlying cells is an immediate result. The feeding scars produced by adult weevils of each species are about equal in depth, although the stem weevil fed more extensively than the seed weevil, as mentioned previously. Moreover, in some sections, a kind of ‘scar’ tissue with elongated cells along the perimeter of the feeding scar was found; this was associated only with adult stem weevil feeding (Figure 8). The explanation for this is not known, but it could be a kind of wound response elicited by stem weevil feeding.

An examination of the histology of cross sections of stem tissue from *T. terrestris* for stem weevil larval feeding damage showed that stem weevil larvae cause extensive feeding damage to pith, xylem, phloem, and woody tissues of the stem. This rampant destruction of tissues by stem weevil larvae, which includes destruction of vital vascular tissues, severely affects the physiology of *T. terrestris*, as demonstrated by the data presented herein. Further consideration of the anatomy of other related genera in the Zygophyllaceae can be found in Metcalfe and Chalk (1950).

No histological assessment was made of seed weevil larval feeding because Kirkland and Goeden (1978a) reported previously that the young larvae fed extensively on the pericarp and interlocular tissues prior to entering the seed chamber, where they also attacked the seeds.

**Plant biomass in groups**

The log biomass of plants harvested from the treatment groups was not significantly different from that of the control group. The average log biomass of the seed weevil and control groups was 6.88 and 6.79, respectively. However, the log biomass of the stem weevil group was significantly lower than that of the seed weevil and control groups, with the log biomass of the stem weevil group averaging 5.46 (Figure 9).
Figure 7. Ninety-five per cent confidence intervals on plant water stress averages (bars of pressure) for both nocturnal (A) and diurnal (B) cycles.
Figure 8. Histological sections of stem of *T. terrestris* showing stem weevil adult and larval feeding damage and plant tissues involved: (A) adult feeding, general; (B) adult feeding, close up showing wound response.
Figure 8 (cont.). (E) control tissue, general. (F) control tissue, close-up.
These results were not unexpected in view of the kind of tissues damaged by stem weevil feeding, especially by the larvae, as compared to the kind of tissues damaged by seed weevil feeding. Data on other parameters, such as rate of growth, also supported these results.

**Seed weevil impact on germination**

Considerable difference was found in the per cent germination of 'fed upon' versus 'not fed upon' carpels from the seed weevil and control groups. In the seed weevil group, four per cent of the 'fed upon' carpels germinated, whereas in the control group nine per cent of the 'fed upon' carpels germinated; 28 per cent of the 'not fed upon' carpels germinated in the seed weevil group versus 46 per cent in the control group (Table 1). The 95 per cent confidence intervals showed significant differences in per cent germination between the 'fed upon' and 'not fed upon' carpels within the seed weevil and control groups and between the 'not fed upon' carpels in the seed weevil and control groups (Figure 10).
Table 1. Percentage germination of 'fed upon' and 'not fed upon' carpels in seed weevil infested versus control groups (100 carpels per replicate).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Carpel condition</th>
<th>Per cent germination</th>
<th>95 per cent</th>
<th>C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed</td>
<td>‘fed upon’</td>
<td>4</td>
<td>1,</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>‘not fed upon’</td>
<td>28</td>
<td>20,</td>
<td>37</td>
</tr>
<tr>
<td>Control</td>
<td>‘fed upon’</td>
<td>9</td>
<td>4,</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>‘not fed upon’</td>
<td>46</td>
<td>36,</td>
<td>56</td>
</tr>
</tbody>
</table>

Figure 10. Ninety-five per cent confidence intervals on per cent germination for ‘fed upon’ and ‘not fed upon’ carpels in seed weevil and control treatment groups.
The germination results obtained with the 'not fed upon' carpels are surprising and striking when it is considered that these carpels, although not damaged externally by seed weevil feeding, exhibited reduced germination. Had feeding occurred, one might have expected the observed decline in germination, but since no external physical damage was apparent on these carpels, this suggests the presence of an intrinsic mechanism in the plant system that is induced by seed weevil feeding or larval development, the latter being the most likely to have stimulated the observed changes in per cent germination of the carpels.

Weevil interaction
An average of 4.2 emergence holes/50 cm of stem length were found in the seed weevil group versus 12.7 for the stem weevil group.

The per cent of carpels infested with seed weevils was 63 per cent for the seed weevil group and 42 per cent for the stem weevil group (Table 2).

Table 2. Statistical treatment of the degree of seed and stem weevil dispersal and/or interference during the study.

<table>
<thead>
<tr>
<th>Stem weevil dispersal (emergence holes)</th>
<th>Mean</th>
<th>95 per cent</th>
<th>C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed</td>
<td>4.2</td>
<td>2.6,</td>
<td>5.8</td>
</tr>
<tr>
<td>Stem</td>
<td>12.7</td>
<td>10.0,</td>
<td>15.5</td>
</tr>
<tr>
<td>Control</td>
<td>0.6</td>
<td>1.2*</td>
<td></td>
</tr>
</tbody>
</table>

*upper limit (one-tailed)

<table>
<thead>
<tr>
<th>Seed weevil dispersal (per cent carpels infested)</th>
<th>Fraction infested</th>
<th>per cent</th>
<th>95 per cent</th>
<th>C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed</td>
<td>35/56</td>
<td>63</td>
<td>50,</td>
<td>75</td>
</tr>
<tr>
<td>Stem</td>
<td>25/60</td>
<td>42</td>
<td>30,</td>
<td>54</td>
</tr>
<tr>
<td>Control</td>
<td>13/56</td>
<td>23</td>
<td>13,</td>
<td>35</td>
</tr>
</tbody>
</table>

The 95 per cent confidence intervals showed that although there was some seed weevil emergence from sampled carpels from all treatment areas, the interference is small enough as evidence by Figures 11A and 11B that significance in other parameters can be attributed to the treatments. Likewise, the presence of the stem weevil in the other treatment areas caused little interference with the results.

These differences were obtained without complete isolation of the groups. If complete isolation had been achieved and larger samples taken, then differences in plant vigour or growth parameters might have been even more striking than those presented here. The fact that enough difference was achieved between treatment groups, without complete isolation, also strongly supports the conclusion that all the results obtained are due to the respective treatment.
Figure 11. Ninety-five per cent confidence intervals: (A) on stem weevil emergence holes (Augs. of plant totals), and (B) on per cent pods infested with seed weevils.
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