Biological Control of the Weedy Plant (*Rumex crispus*) at the Seedling Growth Stage by the Green Dock Beetle (*Gastrophysa viridula*)

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Abstract. In four glasshouse experiments, the effectiveness of the adult green dock beetle *Gastrophysa viridula* (Coleoptera: Chrysomelidae), at the effective number of applied individuals, for use as a biological control agent of curled dock, *Rumex crispus* (Polygonaceae) were studied. The feeding of the beetle was investigated at four different numbers of beetle (0, 1, 2, 3) and at four seedling growth stages of the plant, defined by the average of leaf area per plant (1-1.22, 2-4.45, 3-11.56, and 4-71.52 cm²/plant). Grazing by one, two or three dock beetles did not result in a significant reduction in dock dry weight or shoot numbers at the youngest growth stage. However, both at later seedling growth stages were significantly affected ($P \leq 0.0001$), at any beetles number. The increase of beetle numbers caused nonsignificant increased effect, in some trials, confirming the impact of a single beetle. Three months after beetle grazing, dock seedlings of first, second and third growth stages were not able to regrow, however, some plants at the 4th growth stage, re-emerged. This suggested that the highest effect of beetle's feeding occurs on the early seedling stages. Statistical analysis showed a positive correlation (0.77) between dry weight and shoot number at all the four seedling growth stages, thus confirming the impact of the beetle on both the dry weight and shoot numbers. Combining beetle grazing with other control methods at older dock seedling stages could, therefore, provide better suppression.

Keywords. Curled dock, *Rumex crispus*, Seedlings, Green dock beetle, *Gastrophysa viridula*, Biological control, Regeneration.

1. Introduction

Weedy plants are widely dispersed and persist in arable, rangelands, forests and aquatic ecosystems (Sher and Alyemeny, 2011). In cultivated environments, they have been widely recognised as constraints on agricultural production systems, where they compete with cultivated plants for the available resources needed for growth resulting in negative effects on crop yield and productivity (Strokey, 2006). Resources for which competition occurs include water, nutrients, mineral salts, light, space (Hill, 1977; Zhao *et al.*, 2006; Dunbabin, 2007) and

their requirements have been described as practically the same as the crops with which they cohabit. Weeds population usually persist in both pasture and arable croplands, despite repeated applications of a variety of control methods (e.g., herbicides). Weeds have been shown to be allelopathic (Khanh *et al.*, 2005; Belz *et al.*, 2007; Javaid *et al.*, 2007), depressing growth in desirable plants with toxic residues (Singh *et al.*, 2005). Weeds are also reported to act as banker plants for diverse plant pests (Oudejan, 1994).

Rumex crispus L. (Polygonaceae), curled dock, is widely reported to be one of the most

problematic weed species worldwide. Zaller (2004), reviewed 700 research papers, concentrating on dock ecology and control. He concluded that there are still many aspects of its ecology and, particularly non-chemical, control have yet to be addressed. *Rumex* species are of great importance from the agricultural point of view since they are abundant and exhibit the typical weedy characteristics of competing with sown plants and pasture species for space and resources (Salt and Whittaker, 1998). For example, in Germany, seven of 10 pastoral farms have been heavily contaminated by *Rumex* species (Bach, 1992; Hofmann, 1992) and in Japan, 60 % of grasslands have been reported to be invaded by them (Hongo, 1986). In Europe, 80 % of the herbicides have been estimated to be used in grassland farming are for control of *Rumex* (Gallery, 1989).

The traits which make *R. crispus* and other perennial *Rumex* species so difficult to control include their ability to germinate from seed, combined with massive seed production, and multiple repeat flowerings in any given years. Seed can remain viable in the soil for many years and germinate under different environmental conditions, with quick seedling establishment. The persistent taproot system readily regrows after cutting or from fragments left after damage (Cavers and Harper, 1964; Gwynne and Murray, 1985; Pino *et al.*, 1995; Pye, 2008). Dock seedlings are very widespread and abundant plants in such open habitats (Cavers and Harper, 1964). There are widely reported difficulties in chemical control of these species, which, along with an increasing interest in organic farming practice, those encouraged the use of natural enemies to control them (Martinkova and Honek, 2004). It was reported that releasing of specific biocontrol agents against certain species of weeds can reduce the weeds' adverse effects on crop plants (Harley and Forno, 1992; Julein

and White, 1997). The most thoroughly studied organisms with regard to *Rumex* biocontrol are the Coleoptera, mainly including the green dock beetle *Gastrophysa viridula* (Coleoptera : Chrysomelidae). This beetle is a widespread native of Europe, living in predominantly humid habitats. After eclosion, the larva has three instars before pupation. Its life-cycle takes about 24 days. In the laboratory, five generations per year can be reared, whereas only 2–4 generally occur in the field, depending on the habitat and prevalent environmental conditions (Dagmar *et al.*, 2011). Because of its potential value in the biological control of *Rumex* sp., its biology was studied by Dagmar *et al.*, (2011). However, the use of *G. viridula* for the control of *Rumex* species as weeds, it needs to be combined with other control measures to give total control (Martinkova and Honek, 2004). *G. viridula* beetles have been reported to reduce *Rumex* seed production, affecting its regeneration and hence reducing its adverse impacts, however, even when severely attacked, *Rumex* plants were rarely killed (Zaller, 2004). There have been several studies investigating the effects of the beetle, for instance, on *Rumex* growth, however, no attempts have been made to quantify this effect, particularly during early growth stages of *Rumex*. The present study was carried out to quantify the effects of green dock beetle on *Rumex crispus* at its early growth stages.

2. Materials and Methods

2.1 Growing of *Rumex Crispus* Seedlings for Experimental Trials

Commercial seed samples of *Rumex crispus* (supplied by Hebiseeds, Surrey, UK) were sown in 250 3.5 inch pots at the beginning of September 2014, in a glasshouse at the School of Biological Sciences, University of Reading, UK. Pots were filled with mixed soil of loam, sand and organic

matter at a ratio of 50:25:25%. One seed per pot was buried just beneath the soil surface. Plants were irrigated regularly and NPK (17 %, 11 %, and 10 %) fertilizer, at the rate equivalent to 25 kg ha⁻¹, was added to each pot, two weeks after sowing, to enhance shoot emergence and growth. A number of 112 pots were randomly selected and marked for the definition of plant stage by leaf area measurements. The remaining 138 pots were left for the introduction of beetles, as described below.

2.2 Definition of Plant Developmental Stages

In order to define the leaf stage, the leaf area per plant of 28 seedlings at each of the 4 growth stages were estimated. Leaf area of other 28 seedlings of the same growth stage, was measured also using an image analyser and software (Win DIAS with DIAS 3.2, Delta-T Devices, Cambridge, UK). A linear regression was conducted to identify the following equation for relating estimated (by measurement) to actual (from Win DIAS) areas.

$$Y = B \text{ constant} + B \text{ var } X$$

Where Y = WinDIAS (actual) area, B is a constant (intercept) and X is the estimated area.

The developmental growth stage of plants used in experimental trials were measured prior to each experiment and before beetles' release or use as control, and the leaf area calculated based on the equation above. The developmental growth stages were defined by the determination of the mean of leaf area per plant. Therefore, four selected growth points, were determined by leaf area, representing the seedling developmental stages: 1–1.22 cm²/ plant, 2 – 4.45 cm²/plant, 3 – 11.56 cm²/plant, and 4 – 71.52 cm²/plant.

2.3 Rearing of *Gastrophysa viridula*

Gastrophysa viridula eggs were collected from *Rumex spp* leaves in the field in

wild areas in the north of Wales in August 2014 and reared in the laboratory on moistened filter paper in Petri dishes. The temperature in the laboratory ranged from 22 to 10°C around the 24 hours during the time of beetle rearing. Light was available during the day time. Relative humidity was maintained around 65%. After hatching, larvae were moved to air and light admitting but secure plastic containers. Larvae were fed daily with freshly collected *Rumex obtusifolius* or *Rumex crispus* leaves. When adults emerged from the pupal stage, they were moved to new containers and also fed daily. The cycle was repeated over three months to maintain sufficient stock of the bioagent.

2.4 Releasing *G. viridula* on *R. crispus* seedlings

Experiments were conducted in a greenhouse at 14–22°C and relative humidity ranging between 65–75%. Plants in 7 replicates at each growth developmental stage were randomly infested with either 0 (control), 1, 2 or 3 adult beetles and immediately sealed in a plastic bag. The infestation procedure was repeated on new plants at all 4 target growth points. Sealed, infested plants were placed onto constantly moistened sponge matting, in open plastic trays. Beetles were allowed to graze for 72 hours then returned to their containers. Seedlings were harvested and dry weight and shoot numbers per plant (*i.e.* per pot) were measured and counted. Pots were removed from the trays and watered regularly for three months to monitor long-term reaction after beetle grazing.

2.5 Statistical Analysis

Data from the four experimental stages were combined as the same procedures were followed. One way ANOVA was undertaken using (GENSTAT Version 18, VSN International, and Hemel Hempstead, UK). Mean comparisons were performed by a least

significant difference (LSD) multiple comparison test.

3. Results

3.1 Growth of the Four Seedling Stages of *Rumex crispus*

Table 1 shows the average dry weights ($\text{gm} \cdot \text{plant}^{-1}$) and shoots number/plant for the four growth stages of seedling before each experiment in the absence of beetle infestation (control treatment).

The dry weight (gm/plant) of growth stage 1 was significantly lower ($P \leq 0.02$) than the subsequent growth stages. There was nonsignificant difference in dry weight between stages 2 and 3. However, at stage 4, dry weight was significantly ($P \leq 0.001$) higher than either of the previous growth stages. Shoot numbers/plant at the first growth stage was significantly less than the third and fourth growth stages. However, there was nonsignificant difference in number of shoots/plant between the second, third and fourth stages, suggesting all shoots are produced at the time of emergence or shortly after.

3.2 Effect of Beetle Grazing on the Dry Weight of *R. crispus*

Beetle grazing reduced the mean dry weight of *R. crispus* at all 4 stages of growth (Fig. 1). ANOVA showed significantly ($P \leq 0.001$) lower average dry weight of plants when seedlings were infested with one beetle per plant, but the effect did not increased significantly with increase in numbers of beetles to 2 or 3 per plant.

The percentage of reduction in average of *R. crispus* dry weight ($\text{gm} \cdot \text{plant}^{-1}$) for the 4 growth stages as a result of grazing by 1 or 2 beetles was 44%, compared to the control (0%). Infestation of 3 beetles resulted in the reduction of dry weight ($\text{gm} \cdot \text{plant}^{-1}$) by 73% (Fig. 2). However, this result was

nonsignificant. A single beetle was, therefore, capable of inflicting equivalent damage per plant similar to 2 or 3 beetles.

3.3 Effect of Beetle Grazing on Shoot Numbers of *R. crispus*

The average number of shoots per plant at the combined growth stages was significantly reduced than control at all levels of beetle grazing ($P \leq 0.001$; Fig. 3). The Increase in the number to 2 or 3 beetles*plant⁻¹ caused similar and slightly lower reductions in shoots number, being nonsignificant than that counted by releasing 1 beetle. Infestation of 1 beetle reduced the average number of shoots per plant by 49 % (Fig.4). However, with 2 beetles, the reduction was less (41%) and with 3 beetles it was 58%. The differences were nonsignificant. A single beetle was, therefore, capable of inflicting equivalent damage per plant similar to 2 or 3 beetles.

3.4 Effect of Increasing Number of beetles on the Dry Weight of *R. crispus* at Different Growth Stages

At growth stage 1 (leaf area: 1.22 cm^2), beetle grazing did not significantly reduce the dry weight ($\text{gm} \cdot \text{plant}^{-1}$) (Fig. 5). However, at growth stages 2-4 (leaf areas: 4.45, 11.56 and 71.52 cm^2 , respectively), dry weight was reduced significantly by 1 beetle ($P \leq 0.002$). This effect became more prominent as beetle numbers increased, but not significant.

3.5 Effect of Increasing Number of Beetles on the Number of Shoots of *R. crispus* at the Four Growth Stages

At growth stage 1, (leaf area: 1.22 cm^2) shoot counts per plant were reduced, but the effect was, statistically, nonsignificant, and was equivalent to those occurred by 2 or 3 abundancies of beetles (Fig. 6). However, at stages 2-4 (leaf areas: 4.45, 11.56 and 71.52 cm^2 , respectively), the number of shoots was significantly reduced ($P \leq 0.05$), with,

however, lower effects by 2 beetles at plant stages 2 and 3 than by 1 or 3 beetles. At plant stage 4, there a trend appeared towards greater reduction in shoot numbers as beetle numbers increased, however, the differences were nonsignificant. Hence, at least at stages 1-3, one grazing beetle was found to be effective by reducing the shoot count by an equivalent amount to 2 or 3 beetles.

3.6 Regeneration of *R. crispus* Seedlings After Beetle Grazing

Three months after beetle grazing, the *R. crispus* seedlings grazed by beetles at growth stages 1-3 did not regrow, and their death was confirmed (Table 2). The plants at stage 4 (leaf area: 71.52 cm²), were able to regrow after any tested level of beetle grazing.

Table 1. Table 1. Definition of the developmental stages of *Rumex crispus* by dry weight (gm*plant⁻¹) and shoot count (shoots/plant) at the four growth stages of control plants. * Least significant difference between the means. (Figures with same letter not significant).

Growth stage (average leaf area (cm ²) /plant)	Dry Weight average (gm*plant ⁻¹)	Shoot Count average shoots* plant ⁻¹)
1 (1.22)	0.07 a	3.71 a
2 (4.45)	0.16 b	4.71 ab
3 (11.56)	0.23 b	5.71 b
4 (71.52)	0.43 c	5.57 b
LSD* at 5 %	0.07	1.53

Fig. 1. Effect of the number of beetles on *Rumex crispus* mean dry weight (g*plant⁻¹). Results are combined for all 4 seedling growth stages and shown for four different beetle treatments (0-3 beetles per plant). 0 beetles is the control treatment. LSD (least significant difference) for all means is shown at 5 % significance.

Fig. 2. Percentage (%) of reduction of average dry weight per plant of *Rumex crispus* as a result of four numbers of beetles.

Fig. 3. Effect of the number of beetles on the shoot number of *Rumex crispus* (shoots per pot). LSD (least significant difference) for all means is shown at 5 % significance.

Fig. 4. Percentage (%) of reduction of average shoot numbers per plant of *Rumex crispus* as a result of four numbers of beetles.

Fig. 5. Effect of different number of beetles on *Rumex crispus* dry weight (g*plant-1) at 4 seedling growth stages. Results are shown for 4 different beetle treatments (0-3 beetles/plant) and 4 growth stages. LSD (least significant difference) for all means is shown at 5 % significance.

Fig. 6. Effect of different number of beetles on *Rumex crispus* on shoot numbers per plant of *Rumex crispus*. Results are shown for 4 different beetle treatments (0-3 beetles/plant) and 4 growth stages. LSD (least significant difference) for all means is shown at 5 % significance.

3.6 Regeneration of *R. crispus* Seedlings After Beetle Grazing

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Table 2. Number of re-grown plants of *Rumex crispus* three months after beetle infestation. The shoot count per plant is shown for each of the four growth stages and four levels of beetle grazing. 0 is the control treatment.

Growth stage (average leaf area/plant, cm ²) ↓	Beetle Level →	0 (control)	1	2	3
1 (1.22)		2	0	0	0
2 (4.45)		3	0	0	0
3 (11.56)		3	0	0	0
4 (71.52)		3	2	2	1

3.7. Correlation between Growth Measurements (Dry Weight and Number of Shoots) of *R. crispus*

Statistical analysis showed correlation (Pearson's $r = 0.77$, $P \leq 0.001$), between the dry weight (g*plant⁻¹) of *R. crispus* seedlings and the number of shoots (shoots per plant) at all four growth stages, confirming that as dry

weight decreases, so does the number of shoots. This suggests that beetles grazing on leaves and stems has relatively similar effect and also similar preference.

4. Discussion

The use of biological agents to control the invasive and economically very important weed, *Rumex crispus*, has been investigated since at least the 1970's, however, the present study add to the research to focus on the impacts of the natural dock predator green dock beetle, *Gastrophysa viridula*, at the seedling stage. Results clearly showed that the dock produces multiple shoots at, or immediately post, seed emergence, and if damaged, grazed shoots are not replaced, since no new ones were produced even after 3 months of recovery from beetle grazing. This confirms that feeding at the seedling stage is likely to contribute to the effective control of this species. Green dock beetle feeding at the youngest dock seedling stage tested, showed a nonsignificant reduction. However, this may attributed to the very limited amount of suitable fodder available in such small plants (total surface area 1.22cm²). Intensity of herbivory is known to depend on the intensity and quality of the dock leaves (Renner, 1970).

Thus very small seedlings may provide insufficient substance to sustain grazing, or this may affect the beetle's feeding behaviour. Further research is necessary to explore the reasons for this result, given that at all 3 later seedling stages infection by any number of beetles was found to damage the dock as evidenced by a significant reduction in biomass, suggesting it is an effective biocontrol of seedlings, even at low infestation rates, under laboratory conditions.

Infestation by one beetle reduced the average dry weight of *R. crispus* by 63% and only to 73% with 3 beetles' infestation. Pearson and Brooks (1996) found equivalent effects, with beetle infestation resulting in 70% loss of leaf area of mature *R. obtusifolius*. Beetles in low densities (1-3 beetles), as found in the present laboratory investigation caused, approximately, the same reduction percentage in both dry weight and shoot numbers. Those, could potentially have significant control effects, if infestation took place when *R. crispus* is in early growth stage. Mixed results were achieved, particularly in shoot count reduction, when beetle numbers varied from 1-3, which may be attributed to various factors, such as, the beetles' feeding habits and their condition during the grazing period, or to competition between beetle individuals or microclimatic conditions under the plastic bags. This confirms that further work are needed, under laboratory and field conditions, to determine the optimal releasing rate, and the effects of the available environmental and ecological conditions on the used herbivory.

The competitive ability of mature *R. obtusifolius* plants has also been shown to be affected notably by the beetle *G. viridula* (Cottam *et al.*, 1986). Furthermore, adult beetles were discovered up to 100 m from their releasing point (Naito *et al.*, 1979), suggesting that they are having high potential for *Rumex* spp. control at different growth stages. In the

present study, adult beetle males and females were used. It had been reported that late larval stages can graze more rapaciously than adults (Martinkova & Honek, 2004). The present results indicated sufficient control of dock seedlings at three of the early plant growth stages as *R. crispus* was unable to regenerate until 90 days after the cessation of beetle grazing. But those grazed at the oldest growth stage, some seedlings were later able to regrow. If a longer grazing period could secure sufficient control, may be demonstrated by further work. The rapaciousness of a single beetle was confirmed by its effect on the whole above ground vegetative growth of *R. crispus*, as shown by the correlation (0.77) between the reductions in dry weight and number of shoots. This suggests that, at the early dock growth stages, beetles could potentially kill the seedlings entirely. Hatcher *et al.* (1994) considered that combining beetle grazing with other control methods at older growth stages might provide better results in suppressing docks.

5. Conclusion

Promising results were achieved in this research, particularly the amount of reduction in *Rumex* vegetative growth at seedling stages by beetle grazing, although the beetles were released in low numbers of beetles. Also, the disability of *Rumex* small seedlings to regrow. However, this work needs to be repeated on a larger scale and in the field, to observe beetle behaviour and effectiveness under natural conditions.

- Competing interests

The author declares that he has no competing interests.

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المكافحة الحيوية لأعشاب الحميض في مرحلة البادرات باستخدام حشرة الحميض الخضراء

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المستخلص. في أربع تجارب في البيوت الزجاجية، تمت دراسة فعالية استخدام حشرة الحميض الخضراء وتحديد الأعداد المناسبة منها في مكافحة الحيوية لأعشاب الحميض في مرحلة البادرات. تم تطبيق أربع مستويات من أعداد الحشرة الخضراء (صفر/ نبات: معاملة التحكم - ١/ نبات - ٢/ نبات - ٣/ نبات) على أربع مراحل من مراحل نمو بادرات الحميض تم تحديدها بمعدل مساحة الورقة (١,٢٢ سم^٢ / نبات - ٤,٤٥ سم^٢ / نبات - ١١,٥٦ سم^٢ / نبات - ٧١,٥٢ سم^٢ / نبات). جميع الأعداد المهاجمة من الحشرة (١ - ٢ - ٣) لم تكن ذات تأثير معنوي على مرحلة البادرات الصغرى لأعشاب الحميض (معدل مساحة الورقة (١,٢٢ سم^٢ / نبات) لكن حشرة الحميض الخضراء بأعدادها المختلفة (١ - ٢ - ٣) أحدثت تأثيراً معنوياً عالياً على معدل الوزن الجاف لبادرات الحميض (جم) / نبات ومعدل عدد الأفرع / نبات في بقية مراحل النمو الأخرى (معدل مساحة الورقة: ٤,٤٥ سم^٢ / نبات - معدل مساحة الورقة: ١١,٥٦ سم^٢ / نبات - معدل مساحة الورقة: ٧١,٥٢ سم^٢ / نبات). زيادة أعداد الحشرة إلى ٢ و ٣ / نبات أحدثت تأثيراً أعلى ولكن لم يكن معنوياً وهو ما أكد التأثير العالي معنوياً لحشرة واحدة فقط على نمو بادرات الحميض في مراحل النمو المذكورة. بعد ثلاثة أشهر لم تستطع بادرات المراحل الثلاث الأولى (معدل مساحة الورقة: ١,٢٢ سم^٢ / نبات - ٤,٤٥ سم^٢ / نبات - ١١,٥٦ سم^٢ / نبات) استعادة النمو، مما أكد موتها نتيجة لتأثير الحشرة. بعض بادرات المرحلة الكبرى (معدل مساحة الورقة: ٧١,٥٢ سم^٢ / نبات) استعادت النمو، وهو ما أكد أن تأثير الحشرة الأقوى على نمو بادرات الحميض كان في المراحل الثلاث الصغرى. أوضح التحليل الإحصائي علاقة عالية نسبياً (٠,٧٧) بين بيانات الوزن الجاف وعدد الأفرع في مختلف مراحل نمو بادرات نمو الحميض، ما يؤكد تماثل كبير في تأثير الحشرة على مراحل نمو البادرات الأربع، وكذلك يؤكد الشراهة العالية للحشرة على كامل المادة الخضراء لبادرات الحميض. ومن المتوقع أن يكون تأثير الحشرة الخضراء على بادرات الحميض الكبرى أكبر بمشاركة طرق مكافحة أخرى.