

Growth pattern of diploid and tetraploid spotted knapweed, *Centaurea maculosa* Lam. (Compositae), and effects of the root-mining moth *Agapeta zoegana* (L.) (Lep.: Cochylidae)

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Received 28 April 1987

Revised version accepted 1 July 1988

Summary: Résumé: Zusammenfassung

Glass-faced growth containers were used to study the phenology of three demes of *Centaurea maculosa* Lam. (Compositae), and the impact of root-mining by the moth *Agapeta zoegana* (L.) (Lep.: Cochylidae) on rooting intensity. This moth was recently established in North America for the biological control of spotted and diffuse knapweed, *C. maculosa* and *C. diffusa*. The life cycle, biomass production and longevity of the diploid European host plant (monocarpic biennial) and the tetraploid North American target species (polycarpic perennial) differed considerably, providing a possible explanation for the rapid spread of the tetraploid species in North America. Compared to controls, plants of German and Canadian origin infested by *A. zoegana* showed increased root growth during the mining period and after the emergence of the moths. However, no such difference was found in diploid plants of Hungarian origin. The observed plant reactions partly support, therefore, the hypothesis of a nutritionally controlled response to herbivory. Infested plants did not differ in fecundity compared to controls, but root-mining tends to reduce the survival rate of immature plants. The consequences for the plant population density are discussed.

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Mode de croissance de Centaurea maculosa Lam. (Compositae) diploïdes et tétraploïdes et effets de la teigne Agapeta zoegana (L.) (Lep.: Cochylidae)

Des conteneurs à paroi de verre ont été utilisés pour étudier la phénologie de trois lignées de *Centaurea maculosa* Lam. (composée) et l'impact du minage des racines par la teigne *Agapeta zoegana* (L.) (Lep. Cochylidée) sur l'intensité de l'enracinement. Cette teigne a été récemment introduite en Amérique du Nord pour la lutte biologique contre *C. maculosa* et *C. diffusa*. Le cycle biologique, la production de biomasse et la longévité de la plante hôte diploïde européenne (bisannuelle monocarpique) et des espèces cibles nord américaines (perennes polycarpiques) sont très différents, donnant une explication possible pour la diffusion rapide des espèces tétraploïdes en Amérique du Nord. En comparaison des témoins, les plantes d'origine allemande et canadienne infestées par *A. zoegana* expriment une augmentation de la croissance racinaire pendant la phase de minage et après la sortie des teignes. Cependant, on n'observe pas de telles différences avec des plantes diploïdes d'origine hongroise. Les réactions des plantes confirment en partie l'hypothèse d'un effet nutritionnel de l'attaque. Les plantes infestées ne diffèrent pas des témoins pour la fécondité, mais le minage des racines tend à réduire le niveau de survie des plantes immatures. Les conséquences pour la densité de population des plantes sont discutées.

Wachstumsmuster von diploiden und tetraploiden Gefleckten Flockenblumen, Centaurea maculosa (Compositae), und deren Beeinflussung durch die wurzelminierende Kleinschmetterlingsart Agapeta zoegana (L.) (Lep.: Cochylidae)

Mit Hilfe von Wurzelschaukästen wurden einerseits die Phänologie dreier Taxa der Artengruppe

Centaurea maculosa Lam. (Compositae) untersucht und andererseits der Einfluss der wurzelminierenden Schmetterlingsart *Agapeta zoegana* (L.) (Lep.: Cochyliidae) auf die Bewurzelungsintensität dieser Pflanzen bestimmt. *Agapeta zoegana* wurde kürzlich zur biologischen Bekämpfung von *C. maculosa* und *C. diffusa* in Nordamerika angesiedelt. Die diploide ($2n=18$) europäische Wirtspflanze (monokarp, zweijährig) unterschied sich im Lebenszyklus, der Biomasseproduktion und Lebensdauer deutlich von der tetraploiden ($2n=36$) nordamerikanischen Zielunkrautart (polykarp, kurzlebig perennierend), was eine mögliche Erklärung für die rasche Ausbreitung der tetraploiden Population in Nordamerika liefern könnte. Mit *A. zoegana* infizierte Pflanzen deutscher und kanadischer Herkunft zeigten im Vergleich zu unbefallenen Kontrollpflanzen eine erhöhte Bewurzelungsintensität sowohl während des Minierens wie z. T. auch nach dem Schlüpfen der Falter. Kein diesbezüglicher Unterschied wurde jedoch für die diploiden Pflanzen ungarischer Herkunft gefunden. Die beobachtete Pflanzenreaktion unterstützt daher teilweise die Hypothese einer nährstoffkontrollierten Umverteilung der Assimilate als Folge von Pflanzenfrass. Infizierte Pflanzen zeigten keinen Unterschied in der Fekundität, Wurzelfrass scheint jedoch die Überlebensrate der Rosettenpflanzen zu reduzieren. Auswirkungen auf die Unkrautdichte werden diskutiert.

Introduction

Centaurea maculosa Lam. (Compositae) was introduced into North America from Europe at the turn of the century and has become a serious rangeland weed (Harris & Myers, 1984). The North American *C. maculosa* is almost exclusively a short-lived perennial tetraploid ($2n=36$) that is equated with *C. biebersteinii* DC ssp. *biebersteinii*, a native of East Europe (Dostal, 1976). However, the most widely distributed *C. maculosa* in Europe is the diploid ($2n=18$) biennial *C. maculosa* ssp. *rhenana* (Boreau) Gugler (Dostal, 1976), which was initially thought to be the problem species in North America (Schroeder, 1985). The two species are very similar morphologically. Since 1970, six insect species have been introduced from Europe into North America for the biological control of spotted knapweed and the closely related diffuse

knapweed *C. diffusa* Lam. (Harris & Myers 1984, Müller, 1988).

The root-mining moth *Agapeta zoegana* (L.) (Lep.: Cochyliidae) was one of the candidate agents studied by the Commonwealth Institute of Biological Control as a possible biological control agent for *C. maculosa* in North America (Müller, Schroeder & Gassmann, 1988). It was subsequently cleared for release and became successfully established in British Columbia, Canada and in Montana, U.S.A. in 1984 and 1985 respectively.

The most important feature of a candidate biological control agent, apart from a narrow host range, is its potential impact on the target weed density. However, it is very difficult to predict the potential effect of a phytophagous species prior to its release on the target weed population in the area of introduction (Goeden, 1983; Wapshere, 1985; Müller, 1988); this is especially so when the weed itself has been little studied. A detailed knowledge of the biology of the target species, as well as information on environmental conditions which can act as additional stress factors, are prerequisites for estimating the potential effect of a control agent.

The study of a root-feeding species causes special problems because of its inaccessibility. Compared to the effect of defoliation, very little is known about the impact of root-feeding on parameters of plant fitness (Crawley, 1983; Andersen, 1987). Root-feeding is expected to have both an effect on the uptake of water and nutrients and nutrient accumulation and, as an additional sink, on the partitioning of assimilates within the plant (Brouwer, 1983). The hypothesis of such a nutritionally controlled reaction to herbivory is tested in this study, and the potential of *A. zoegana* as a biological control agent will be discussed. Experiments were designed with two objectives: (1) to study the life cycles and root development of the European host plant in comparison to the target plant, and (2) to investigate the impact of root feeding by *A. zoegana* on the phenology of the host plants.

Numerous types of glass-faced containers have been employed in root research (for a review see Böhm, 1979). They have mainly been used to study root penetration, water and nutrient uptake, and root pathology, as well as for the screening of cultivars and to assess the germination power of seeds. Root observations through glass-faced panels are also useful for the study of

a variety of root/herbivore interactions. This method, however, has seldom been used for this purpose (but see Pitcher & Flegg, 1965).

Materials and methods

Construction, filling and handling of the container

Constructional details are shown in Fig. 1. The container is held together by the tension of conical plastic clamps of the type used by electricians. Considerable care was taken in filling the container. Sand was always added to the soil in order to get a homogeneous bulk density and a

suitable particle size, to enable an adequate water supply throughout the experiment and to avoid subsequent settling of the soil, which could cause tearing of the roots. The sand-soil mixture (different ratios according to the experimental objectives) was allowed to become slightly dry, then sieved through a 1 cm² mesh grid, lightly moistened and transferred into the container in successive layers of 10 cm. Each layer was firmed with a wooden board, the top of each layer being loosened before the next layer was added, to prevent an interface forming.

Filling took approximately 20 min per box. The whole box was then immersed in a barrel of water for 10 min and then left upright for two weeks before the beginning of the experiment. Subsequent watering was done only from above.

Several seeds of uniform size were sown in the container and after two weeks the seedlings were reduced to two individuals with comparable root length. The boxes were inclined at an angle of 10° from the vertical, with the glass side downwards, so that the tap roots touched the glass shortly after germination and grew directly along the glass wall (Fig. 2). The boxes were kept in half-shade during the experiment and the position adjusted every two weeks. Both sides of the box

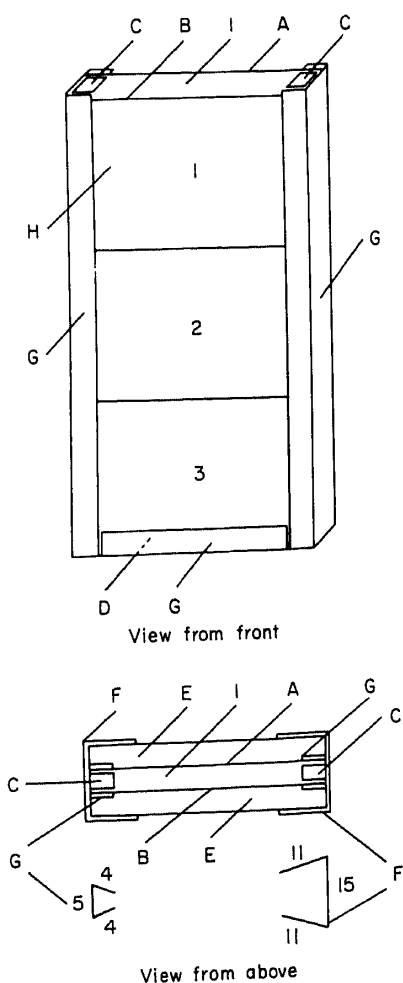


Fig. 1 Components of the glass-faced growth container. (A) support plate (e.g. eternite), 0.5 × 50 × 100 cm high; (B) glass plate, 0.2 × 50 × 100 cm high; (C) wood (vertical support), 3.5 × 2.5 × 100 cm high (two pieces); (D) wood (bottom support with three drainage holes), 3.5 × 3.5 × 44 cm long; (E) styrofoam sheet, 5 × 50 × 100 cm high (two pieces); (F) metal clamps (for styrofoam sheets), 4 cm wide (four pieces); (G) hardened PVC clamps for support and glass plate (two pieces 100 cm long, and one piece 40 cm long); (H) acetate sheet (divided into three sectors), 42 × 96 cm; (I) sand-soil mixture.

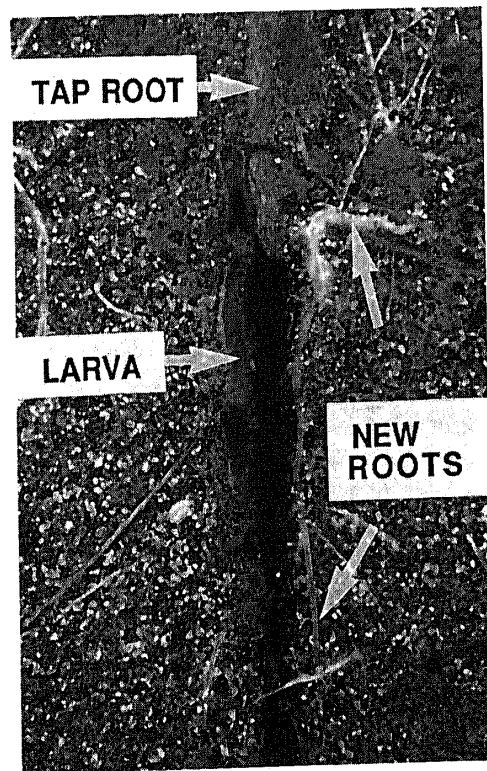


Fig. 2 Local root length increase as a reaction to mining by *A. zoegana*.

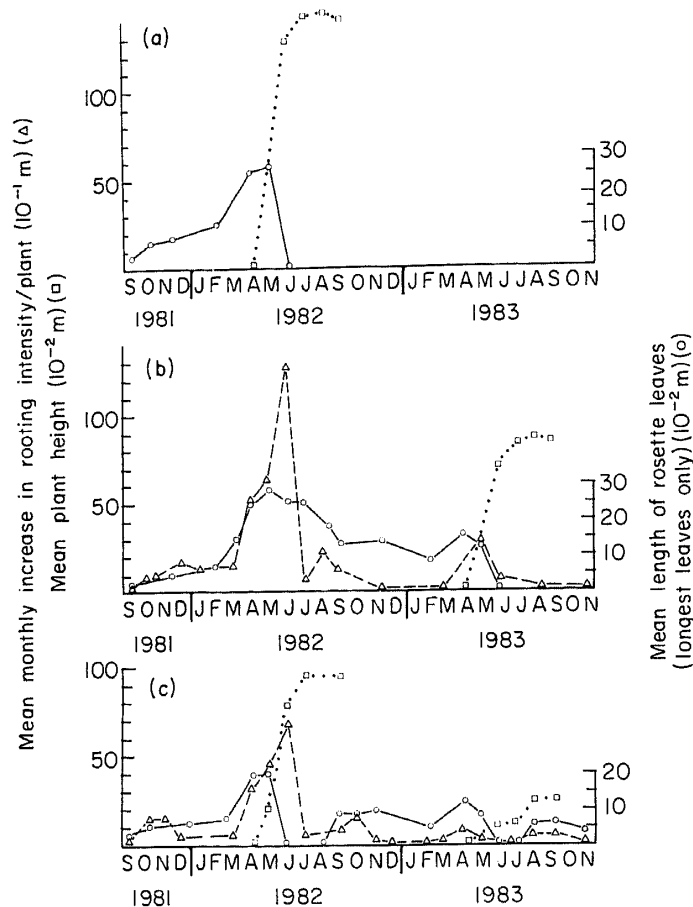


Fig. 3 Synopsis of phenological development of diploid and tetraploid *C. maculosa* plants. (a) *C. maculosa* Hungary ($2n=18$), flowered in the first year ($N=6$); (b) *C. maculosa* Hungary ($2n=18$), flowered in the second year ($N=4$); (c) *C. maculosa* Canada ($2n=36$) ($N=5$). Rooting intensity = root length visible/ 0.375 m^2 ; values for rooting intensity could not be measured separately for (a) and (b) as both flowering and rosette plants occurred in the same container (average values are shown in (b)).

were closed with a styropor plate (5 cm thick) to protect the roots from light and to provide temperature insulation. These plates were only removed to monitor the development of the herbivores and the roots, and to record root growth.

Recording of root development

To monitor the dynamics of root development, rooting intensity, here defined as the length of new roots visible per viewing surface, was measured monthly (cf. Böhm 1979, for a general

discussion of root parameters). A transparent acetate sheet was placed on the glass. The newly formed roots, which are easily distinguishable from older roots by their lighter colour, were traced with a waterproof felt tip pen onto the acetate sheet. Different colours were used for each control date and the root length increase was later recorded by means of an opisometer (rotating wheel).

Experiments

The above technique was used to describe root growth of plants grown under the following

Table 1 Yield parameters of uninfested diploid, Hungarian and tetraploid, Canadian *C. maculosa* plants

		2n = 18 (Hungary)		2n = 36 (Canada)	
		1982	1983	1982	1983
Dry weight (g/plant)	\bar{x}		7.71		2.93
	SD		1.46		2.10
	N		10		5
Basal diameter of shoots (mm)	\bar{x}	8.3	4.5	6.2	2.08
	SD	0.8	1.69	1.8	1.50
	N	6	4	5	5
Seeds/flowerhead	\bar{x}	19.7	18.7	15.2	7.4
	SD	10.3		7.9	
	N	60	373	140	474
Flowerheads/plant	\bar{x}	90.7	93.25	55.3	37.7
	SD	28.6	9.54	28.8	21.36
	N	6	4	5	5
Seeds/plant/year with flowers	\bar{x}	1787	1635	840	228*
	min-max	1184-3381	1492-1721	156-1540	20-537
	N	6	4	5	5
Seeds/plant	\bar{x}		1726		1022†
	min-max		1184-3381		216-1765
	N		16		15

Differences between diploid and corresponding tetraploid plants are all significant at 5% level (Mann-Whitney test), except seeds/plant.

* Low seed production in the second year probably due to the reduced rooting volume in the growth container.

† Plants still alive, when experiment was stopped.

conditions. Diploid *C. maculosa* ssp. *rhenana* ($2n=18$) and tetraploid *C. maculosa* (*C. biebersteinii* ssp. *biebersteinii*, $2n=36$) were sown separately in eight growth containers on 8 August 1981, and later reduced to two seedlings per box. Two diploid and one tetraploid plant died for unknown reasons as young rosettes. Three first instar larvae of *A. zoegana* were transferred between end the July and early August 1982 onto the bases of the rosette leaves of each of 10 tetraploid and 4 diploid plants. The larvae mine spirally, beneath a silken web, in the cortical tissue of the tap root (Fig. 2).

In a second experiment, diploid plants of German origin were sown on 15 March 1982, in two different soil types (organic/nutrient rich and sandy/nutrient poor; cf. Müller, 1984, for data on soil analyses). Nine plants in each soil type were infested with *A. zoegana* larvae in early August 1982 and as described for experiment 1, and 5 plants in each soil type were used as controls. Flowering plants were cut in November 1982 and 1983, and morphological parameters and dry weight measured. In order to assess seed production, mature flower heads were removed sequentially shortly before seed spread. The relation between seeding date and the probability of

flowering has also been studied with plants, grown in one litre pots and a standardized soil mixture, such as used in experiment 1.

Results

Diploid/tetraploid plant comparison

The phenological development of selected parameters of diploid (host plant) and tetraploid (target plant) *C. maculosa* plants is shown in Fig. 3. The growth rate of both the rosette leaves and the roots of the two knapweeds increased in spring in both years at approximately the same time. In both knapweeds shoot growth started about one month later and shortly afterwards the rosette leaves began to dry up. Root growth decreased drastically following formation of the flower buds, i.e. shortly before the maximum shoot length was reached. The generally lower growth rates for the tetraploids in the second year (Table 1) were probably due to the decreased rooting volume available in the containers after one year's growth.

The two knapweeds showed distinct differences in their life cycles. The tetraploid Canadian plants were all polycarpic perennials, producing only one shoot in the first year and 2-7 shoots in

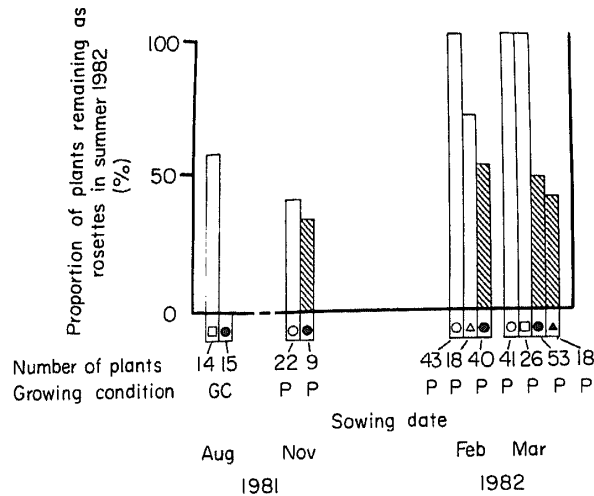


Fig. 4 Time of flowering in relation to sowing date for several *C. maculosa* populations. Origins of seeds: □ diploid ($2n=18$); ○ Germany, △ Austria, □ Hungary; ▨ tetraploid ($2n=36$); ● Canada, ▲ Rumania. P=potted (one plant per pot; 700 cm³ of sand-soil mixture); GC=growth container.

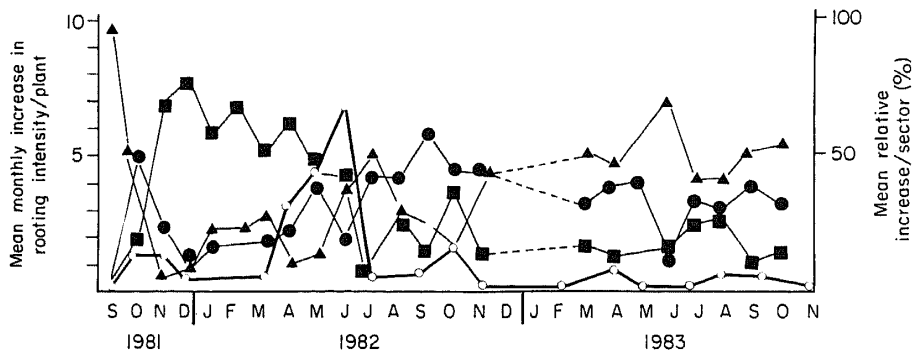


Fig. 5 Monthly rooting intensities relative to sectors of the root container for tetraploid *C. maculosa* (Canada); ▲ sector 1 (uppermost third of the box), ● sector 2, ■ sector 3, ○ length in metres.

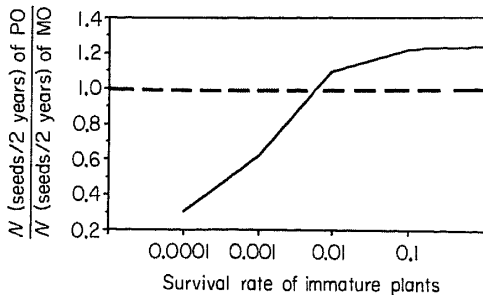


Fig. 6 Effect of different survival rates of immature plants on seed production in 2 years for polycarpic Canadian ($2n=36$) and monocarpic Hungarian ($2n=18$) *C. maculosa* plants. $(NPO/2 \text{ years}) / (NMO/2 \text{ years}) = [(NPO1 \times S) / (NPO1 + (NPO2) / [(NMO1 \times S \times P)(P) + NMO2(1 - P)])]$. N: number of seeds; PO1: polycarpic plant, first year; MO2: monocarpic plant, second year; S: survival of immature plants; P: probability of flowering (seed production) in the first summer after seeding ($=0.43$).

the second year, whereas six of the diploid Hungarian plants flowered in the first year and the remaining four in the second year. All diploid plants produced only one shoot and died after flowering (monocarpic). Diploid plants of Hungarian origin that flowered in the first year had significantly larger root diameters in the previous winter than those that remained in the rosette stage (Müller, 1984).

A higher proportion of potted tetraploid *C. maculosa* bolted in comparison to the potted diploids, when sown in early spring, but no distinct difference was observed when sown in late autumn (Fig. 4). Hence, plants grown from tetraploid seeds of Rumanian origin (H. Müller, unpubl.) showed a pattern similar to the Canadian tetraploids, indicating a possible genetic basis for this trait in the tetraploid plants (Fig. 4).

All the tap roots had reached a minimum

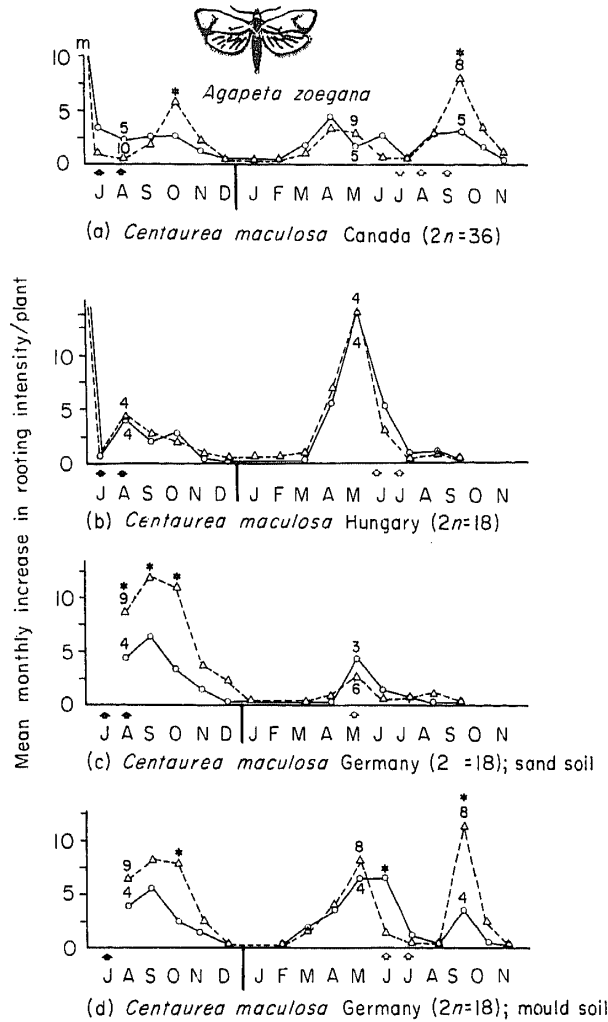


Fig. 7 Effect of root herbivory by *A. zoegana* on rooting intensity in the upper third (first sector) of the root container. (a)+(b) sown 8 August 1981, (c)+(d) sown on 15 March 1982. Y-axis: rooting intensity is measured by root length visible per 0.125 m²; X-axis: months of 1982/83. —▲— infested plants; —○— control plants; —○— number of surviving plants; ◆ transfer of first instar larvae; ⬆ emergence of moths; * statistical difference between infested and control plants at the 5% level (Mann-Whitney *U*-test, two sided); cumulative rooting intensity did not differ between control and infested plants at the time of infestation.

length of 80 cm just two months after sowing. In the first year the tetraploid plants had reached only 67% of the sum of the monthly rooting intensity scores attained by diploid plants (12% in the first sector, i.e. in the uppermost third, 57% in the second sector and 57% in the third sector). The relative rooting intensities in the three sectors is shown in Fig. 5 for the tetraploid plants. Similar trends were found for all of the test plants.

Yield as well as annual production of above-ground tissue of the diploid plants was generally

higher than that of the tetraploids (Fig. 3, Table 1). However, life-time fecundity may be similar due to the polycarpic cycle of the tetraploid species.

The observed values for seed production and for the proportion of flowering plants in the first year (Fig. 3, Table 1) were used to estimate the effect of different survival rates of immature plants on the fecundity ratio between polycarpic Canadian and monocarpic Hungarian plants (Harper, 1977). This crude model (Fig. 6) indi-

cates a higher fitness for perennial plants when plant survival is high (e.g. when colonizing disturbed habitats), but an advantage for the monocarpics (delayed flowering) when plant survival is low (e.g. in stable, high density conditions).

Effect of the root mining on plant performance

The emergence rates of adult moths, 40% and 42% respectively, are similar to results of rearings on potted plants.

No significant differences in life cycle, phenology and growth intensity of individual plant parts, nor in seed production between infested and uninfested plants, were found for the two knapweeds. However, two of the ten tetraploid and one of the four diploid plants infested by *A. zoegana* died prematurely, indicating that infested plants may be killed by the larvae. All of the nine control plants survived.

Root growth in the upper 30 cm was analysed separately since all mining occurred in this sector (Fig. 7). Compared to the control plants, infested tetraploid plants showed a significant increase in root growth about 3 months after larval transfer, whereas no difference was detected in the diploid plants of Hungarian origin (Fig. 7a, b). The same experiments with diploid plants of German origin produced a pattern different from the diploid Hungarian plants (sown in autumn 1981), but similar to the tetraploid North American plants (Fig. 7c, d), i.e. an increased rooting intensity when infested.

Plants growing in the sandy/nutrient poor soil showed reduced growth, retarded development and higher mortality compared to plants growing in organic/nutrient rich soils. Infested plants were generally smaller than the corresponding controls, but seed production was not reduced (Fig. 7c, d; Müller, 1984).

Compared to the control plants, some infested plants showed increased rooting intensity even after emergence of the moths (Fig. 7a, b). Figure 2 illustrates that overcompensation occurred in the direct vicinity of the mining activity. The results demonstrate that under the test conditions some roots were able to compensate for damage inflicted during both the mining period and the attack-free period following emergence of the moths.

Discussion

As with root studies in general, the method is relatively time consuming and did not permit the number of replicates required for detailed statistical analysis, and so provides only a primarily qualitative comparison.

The diploid Hungarian plants differed greatly from the tetraploid North American plants in their life cycle and longevity. The potential for high seed production early in life (Fig. 6), together with the perennial life cycle and the smaller plant biomass, may be responsible for the rapid spread and the competitive power of the tetraploid species in overgrazed North American rangeland.

According to Brouwer (1983) plant responses to herbivory are best explained by nutritional control. Root pruning leads to reduced water and mineral absorption, which increases the water potential gradient. The resulting carbohydrate accumulation, due to a reduced leaf extension rate, will lead to an increased relative growth rate of the remaining roots (Brouwer, 1983; McNaughton, 1983). The enhanced rooting intensity of some of the infested *C. maculosa* plants supports this hypothesis.

Additional stress factors, such as plant competition or adverse environmental conditions (e.g. drought) may, however, reduce or suppress compensation (Crawley, 1983). Experiments to study the joint effects of root herbivores, plant competition and nutrient supply on resource allocation and population ecology of diploid *C. maculosa* are presently being carried out.

The fact that diploid plants of Hungarian origin showed no increase in root growth after herbivory may be due to their larger root biomass at the time of infestation (Figs 3, 7). A genetically based difference in resource allocation patterns between the diploid and tetraploid populations, as well as between the two diploid ones, may be a further explanation for the observed plant reaction after herbivory.

Although root mining did not affect fecundity, survival of immature plants seems to be reduced compared to control plants, as has been shown with potted plants (Müller, Schroeder & Gassman, 1988). A recently developed plant population model indicates that the survival rate of rosettes is most important in determining equilibrium knapweed density in Canada (J. Myers, pers. comm.). *Agapeta zoegana* will therefore be especially promising as a biological control agent

(Müller, Schroeder & Gassman, 1988; Müller, 1988).

The method described is a helpful tool in biological weed control projects involving root herbivores. It allows: (1) study of the impact on plant parameters at different levels of herbivore loads in combination with additional stress factors (e.g. soil type, plant competition, water and nutrient shortage); (2) investigation of feeding behaviour and the phenology of the attack; and (3) collection of information on intra- and inter-specific competition between candidate biocontrol agents.

Acknowledgment

I wish to express my thanks to D. Schroeder for his encouragement, J. Bockemühl for technical advice, to H. Rowell, M. Rowell-Rahier, D. Schroeder, C. S. A. Stinson, J. K. Waage and H. Zwölfer for useful comments on earlier drafts of this manuscript, and to K. Hurlle for the critical review. Thanks also to H. Krummenauer and J. Frantzen for technical assistance and I. Singh for preparing the figures and photographs. The study was supported by the Canadian Department of Agriculture and a grant from the Swiss Government.

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