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The Search for Effective Biological Control Agents in Europe 1. Diffuse and Spotted Knapweed

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Abstract

The difficulties experienced during foreign exploration in the search for safe and effective biological control agents are discussed for diffuse and spotted knapweed (*Centaurea diffusa* and *C. maculosa*, respectively). Apart from unsolved taxonomic problems in case of spotted knapweed, field surveys were handicapped by the fact that the optimal survey area was not accessible and that both target weed species were of scattered occurrence in the marginal areas surveyed. Well-developed complexes of specific natural enemies were not found and the effect of individual species on the distribution and abundance of their host plants could not be studied in the field because of low densities of plants and natural enemies. However, the field surveys were still rewarding. Information is given on 23 species of potential control agents, six of which have already been released in North America. Attention is called to the increasing demands for safety specifications for potential control agents of diffuse and spotted knapweed. It is proposed to assess these demands carefully.

Choix d'Agents Efficaces de Lutte Biologique — Possibilités et Facteurs Limitatifs Généraux

Compte tenu du temps et des sommes qu'il faut consacrer aux études sur le terrain et aux essais de spécificité d'hôtes, on propose de choisir des agents biologiques apparemment efficaces après l'étude de la documentation générale et des enquêtes sur le terrain dans des régions appropriées. Le rapport traite des difficultés suscitées à cet égard par les chercheurs ailleurs en Europe et cite comme exemple les recherches sur *Euphorbia* et *Centaurea*. On accorde une attention particulière aux aspects tels l'accessibilité de zones d'étude appropriées, la répartition et l'abondance des plantes hôtes, la présence et l'abondance d'organismes bien adaptés pouvant potentiellement jouer le rôle d'agents biologiques ainsi que les problèmes techniques qui se posent. L'auteur montre qu'il est souvent difficile de déterminer l'efficacité des organismes associés à la plante nuisible cible dans son aire de répartition indigène ou de recueillir un nombre suffisant d'échantillons en vue des études en laboratoire. En outre, il parle de certains problèmes qui se posent dans le choix d'agents biologiques potentiellement efficaces, comme l'aire de distribution des plantes hôtes, les conflits d'intérêts en ce qui concerne le lieu d'introduction et la question des espèces de plantes indigènes rares et menacées de disparition. Il précise qu'il faut parfois éliminer certains agents biologiques appropriés et efficaces en raison de facteurs limitatifs inhérents à la lutte biologique.

Introduction

Alien weeds, established by accident or design, usually are introduced without their specific natural enemies, i.e. phytophagous arthropods and phytopathogens (Goeden 1971, 1974; Maw 1976). Foreign exploration in the weed's native range for suitable and effective control agents is therefore a major part of each classical biological control project. Before foreign exploration is started, it is indispensible to determine the identity and taxonomic position of the target weed, collect information on its origin and the history of its introduction and distribution, as well as on the arthropods and pathogens associated with the weed throughout the colonized area.

Surveys for safe and potentially effective biological control agents should preferably be carried out in the area of greatest diversification of the genus or subgenus to which the target weed belongs (Wapshere 1974*a*, 1981). A second guideline for selecting the survey area is that it should be climatically similar to the region where the weed occurs at noxious levels (Wapshere 1981).

Another important consideration in foreign exploration is the early selection of potentially effective control agents. Proposals on how these agents can be selected are given by Harris (1973), Wapshere (1974*b*), and Goeden (1983).

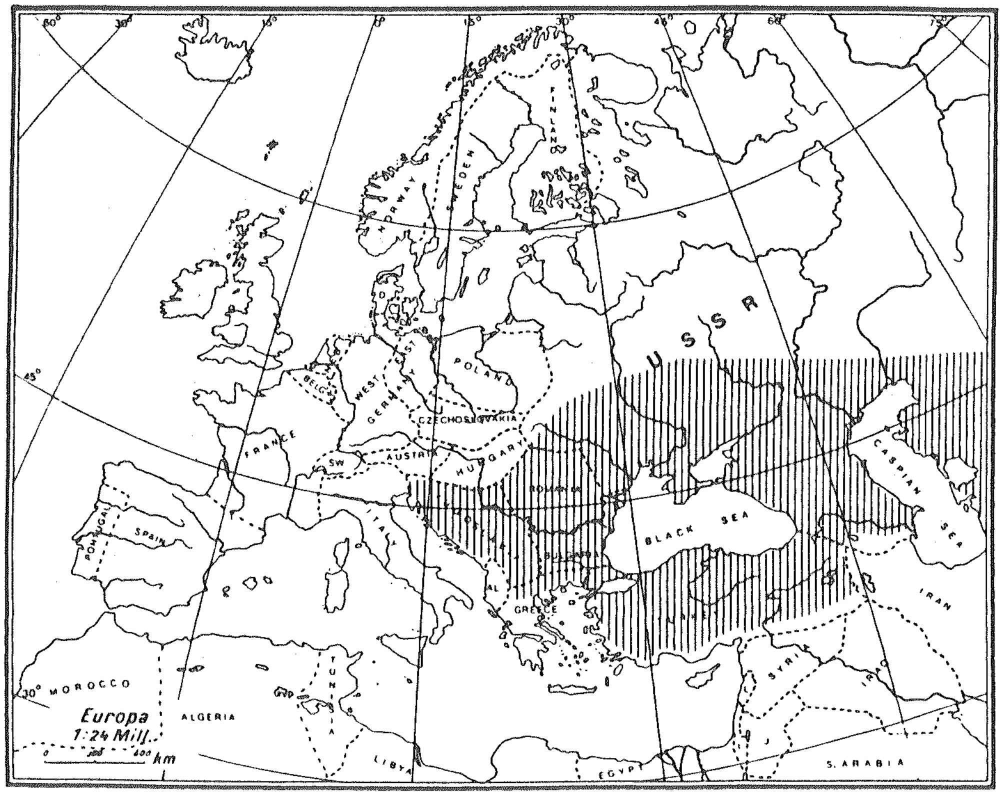


Fig. 1. Geographic distribution of *Centaurea diffusa* Lam. in Europe (Dostal 1976).

Some of the difficulties experienced during foreign exploration to follow these scientifically sound demands will be illustrated and discussed in this paper, using work in Europe on diffuse and spotted knapweed as examples.

The Taxonomy of Diffuse and Spotted Knapweed

The genus *Centaurea* (Compositae) consists of around 500 predominantly Mediterranean species. Diffuse knapweed, *Centaurea diffusa* Lam., is taxonomically well defined, and North American and European plants seem to belong to the same species. The spotted knapweed of North America is usually identified as *C. maculosa* Lam. (Moore and Frankton 1974). *C. maculosa* belongs to the difficult and poorly studied *C. paniculata* L. group of about 30 species with many transitional forms and hybrids

(Landoldt *et al.* 1972). There is little consensus how this group should be treated and the specific characters used are poorly defined and plastic. The treatment by Dostal (1976) lists 13 species and 15 subspecies in the section Maculosae that are widely distributed over Europe. *C. maculosa* in this treatment is a diploid ($2n = 18$) biennial found from central France to south Germany and northern Italy. The North American spotted knapweed is a perennial tetraploid ($2n = 36$) and is found in a much drier climate. Moore and Frankton (1974) equated the North American species with *C. stoebe* L. and *C. maculosa* ssp. *rhenana* (Boreau) Gugler which is treated by Dostal (1976) as *C. rhenana* Boreau. It is also a diploid ($2n = 18$) biennial extending from southeast Europe to central Russia. The best fit in Flora Europaea (Dostal 1976) is *C. biebersteinii* DC., which is a biennial or perennial tetraploid ($2n = 36$) that occurs in Albania, Bulgaria, Czechoslovakia, Hungary, Romania and Russia. Plants collected in this region by CIBC were identified as *C. micranthos* S.G. Gmeln ex Hayek or *C. maculosa* var. *micranthos*. This according to Dostal (1976) is a synonym for *C. biebersteinii* ssp. *biebersteinii*. However, all the plants collected from the region (mainly Hungary) were diploid ($2n = 18$) except for those from the Botanic Gardens, Cluj (Romania) which were tetraploid ($2n = 36$). It is now almost certain that because of different taxonomic treatments of spotted knapweed in Europe and North America, the problem species of British Columbia and the northwestern States of the United States has been largely missed in the surveys done in Europe for potential biological control agents.

The Selection of Suitable Survey Areas

The Distribution of Diffuse and Spotted Knapweed

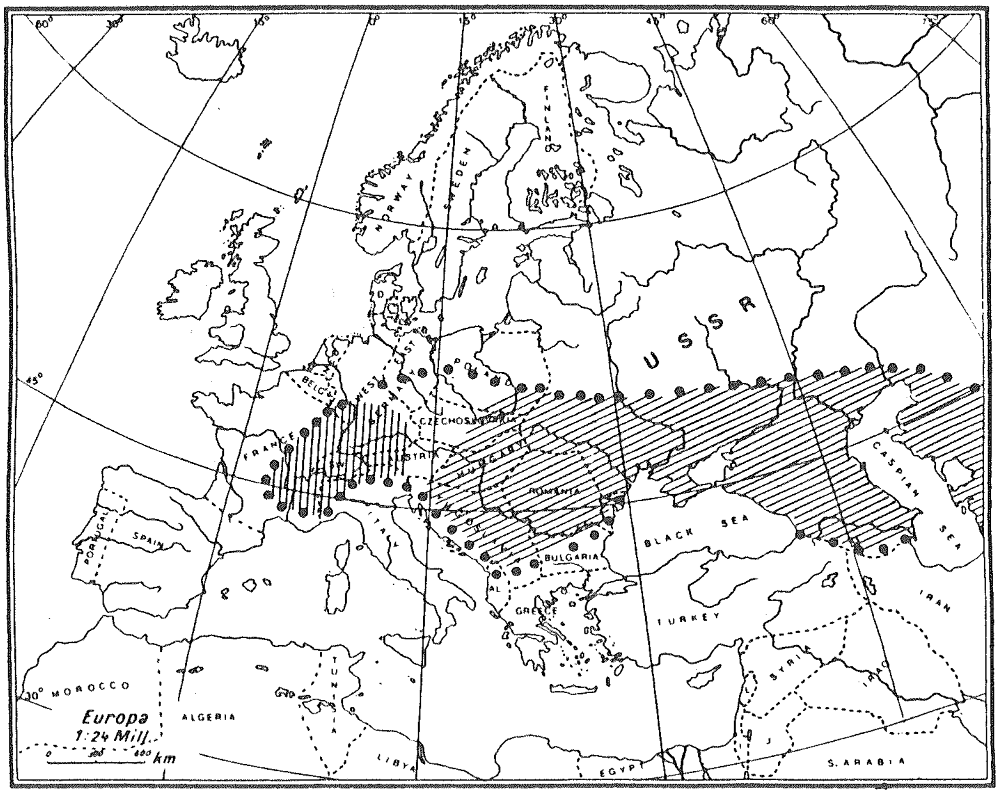
The present distribution of *C. diffusa* in Europe and western Asia is shown in Fig. 1. Diffuse knapweed is a typical plant of the steppe vegetation in southern Russia and western Asia. Popova (1960) reported that in the Crimea it is a serious problem in dryland alfalfa, where it comprises 40–65% of the stands. West of the USSR it is only common and relatively abundant in the former steppe area in Dobrugea (Romania) and in northwestern Turkey. In Bulgaria, northern Greece and Yugoslavia it occurs as widely scattered patches. Small naturalized stands occur along the Adriatic coast of Yugoslavia, in southern France (Apt) and southwestern Germany (Karlsruhe).

In North America, the species was first recorded in Washington State in 1907 (Howell 1959) from where it spread into British Columbia (Groh 1943). Presently diffuse knapweed infests some 26 000 ha in British Columbia, as well as small areas in Alberta, Saskatchewan and Manitoba (Harris and Myers 1984). In the United States, *C. diffusa* occurs in Montana, Washington, Idaho, Oregon and California (Maddox 1982). It is suspected that first introductions were with alfalfa seed from Turkmenistan, USSR.

The distribution of the Eurasian *C. maculosa* group of species is given in Fig. 2. According to Dostal (1976) *C. rhenana* is the most widely distributed species, followed by *C. biebersteinii* ssp. *biebersteinii*, whilst *C. maculosa* spp. *maculosa* occurs only within the western part of the *C. rhenana* distribution range. A fourth species, *C. vallesiaca* (DC.) Jordan, is locally found in the western Alps. With the exception of some areas in eastern Austria and northwestern Hungary, spotted knapweed occurs in low density as widely scattered patches throughout its European distribution area. No information is available on the occurrence and abundance of spotted knapweed(s), especially of the tetraploid *C. biebersteinii* ssp. *biebersteinii*, in the USSR. All western collections of this species are diploid, with the exception of plants, although of unknown origin, in the Botanic Gardens Cluj (Romania). It cannot be excluded that the tetraploid *C.*

biebersteinii ssp. *biebersteinii* is restricted in distribution to the southern USSR, and the seeds first imported accidentally into North America originated from this area.

Groh (1943) reported that spotted knapweed was first collected in North America at Victoria, British Columbia in 1883. At present it infests some 2 500 ha in British Columbia, is common in Ontario, Quebec, and the Maritimes (Frankton and Mulligan 1970), and there are some small stands in Alberta (Watson and Renney 1974). However, the main infestations of more than 1 million ha are in Montana (800 000 ha), Idaho, Washington and Oregon, U.S.A. (Maddox 1982).



C.maculosa ssp.maculosa (C.stoebe ssp. maculosa (Lam)

C.biebersteinii ssp.biebersteinii (C.micranthos S.G.Gmelin ex Hayek)

C.rhenana Boreau

Fig. 2. Geographic distribution of 3 taxa of the *Centaurea maculosa* group of species in Europe (Dostal 1976).

Based on the above information and the generally accepted guidelines for the selection of optimal survey areas, surveys for safe and potentially effective control agents for both diffuse and spotted knapweed should have been started in the southern USSR. This is also indicated by a comparison of the climatic diagrams (Walter and Lieth 1960) for the southern USSR and most of the area infested by the two knapweeds in North America. The climate in areas west of the Russian border differs in precipitation and temperature from the climate in most North American knapweed infestations. An exception is the Dobrugea area in eastern Romania which has an almost identical climate to the greater part of the area infested by spotted knapweed in Montana, U.S.A.

Field Surveys in Europe

Surveys by the European Station, CIBC, were started in 1961 in the western part of the European *C. maculosa* (*C. rhenana*) distribution area. This was justified for the following reasons: (*a*) the apparently optimal survey area in southern USSR was not accessible (which still applies); (*b*) the North American spotted knapweed was equated with *C. maculosa* ssp. *rhenana*; and (*c*) because of certain financial constraints. Between 1961 and 1964 some 40 *C. maculosa* sites were surveyed in France, Switzerland, southern Germany and eastern Austria (Zwölfer 1965). From 1965 onwards the survey was extended to eastern central Europe (Czechoslovakia and Hungary) and the Balkans (Yugoslavia, northern Greece, western Turkey, Bulgaria and Romania) to include the European distribution of *C. diffusa*.

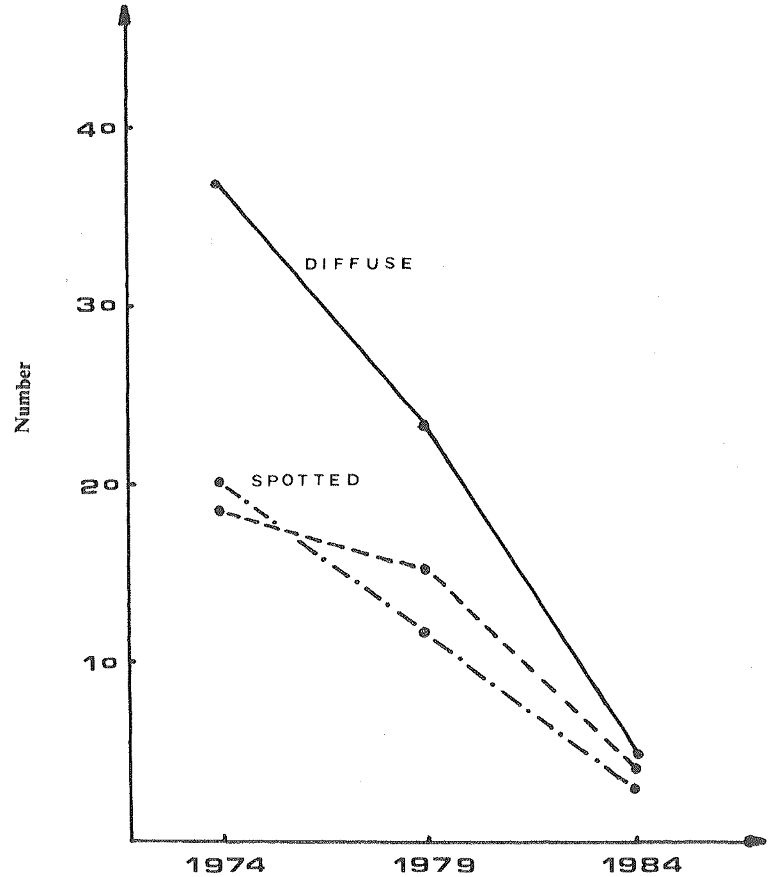


Fig. 3. Number of diffuse and spotted knapweed sites in western, central and eastern Europe, 1974–1984.

It was found that diffuse and spotted knapweed in Europe predominantly occur on ruderal sites (wasteland, gravel pits, quarries, roadsides, embankments, etc.) and only rarely in semi-natural habitats, such as dry, south-exposed slopes in vine-growing areas (*C. maculosa*) or permanent sheep pasture on shallow, gravel soils (*C. diffusa*). Although plant density and total number of plants were low at many of the ruderal sites, both knapweeds were relatively common in most of the survey areas when the survey was started. As shown in Fig. 3, this has drastically changed during the past 10 years. In addition to the decrease in number of sites, plant density has also decreased and stands with more than 500 plants are actually very rare.

Table 1. List of specific and oligophagous biotic agents attacking *Centaurea* spp. in Europe.

Biotic agent	Part attacked	*C. maculosa*	*C. diffusa*	Other *Centaurea* spp.
Fungi, Pucciniaceae				
Puccinia centaureae DC.	L	+	+	○
P. c. var. *diffusae* Savile	L	–	○	–
P. jaceae Otth.	L	+	+	○
P. j. var. *diffusae* Savile	L	–	○	–
Acarina, Eriophyidae				
Aceria centaureae (Nal.)	L	+	+	○
A. grandis (Nal.)	F	+	–	○
Aphidoidea, Aphididae				
Chomaphis centaureae CB.	R	○	–	○
Dactynotus jaceae (L.) ssp.				
D. reticulata HRL.	Sh(L)	○	–	–
Macrosiphoniella stägeri HRL.	Sh(L)	○	–	–
Protaphis alexandrea (Nevs)	Sh(L)	○	+	○
Trama centaureae CB.	R	○	+	○
Lepidoptera, Tortricidae				
Epiblema scutulana (Den. & Schiff.)*	F/Sh	○	–	○
Pelochrista medullana Zeller	R	+	+	○
Lepidoptera, Cochylidae				
Agapeta zoegana L.	R	+	–	○
Cochylis posterana Zeller*	F/Se	+	–	○
Stenodes alternana (Steph.)	F/Se	+	+	○
S. meridiana Stgr.	R	+	–	○
S. perfusana (Guen.)	F/Se	○	–	○
S. straminea (Haw.)	F/Sh	+	+	○
Lepidoptera, Gelechiidae				
Depressaria arenella Schiff.*	L	–	+	○
Metzneria diffusella Englert	F/Se	–	+	–
M. paucipunctella Zeller	F/Se	+	–	–
Pterolonche inspersa Stgr.	R	+	+	–
Lepidoptera, Nymphalidae				
Melitea ?phoebe Knoch*	L	–	+	○
Lepidoptera, Momphidae				
Pyroderces agyrogrammos Zeller*	F/Se	+	+	+
Diptera, Agromyzidae				
?*Melanagromyza aeneiventris* (Fallén)*	Sh/R	–	+	○
Diptera, Anthomyiidae				
Pegohylemyia centaureae Hennig	CB	+	+	–
Diptera, Cecidomyiidae				
Loewiola centaureae (F.Lw.)	L	○	–	○
Clinodiplosis cilicrus Kieff.	F	–	+	–
Diptera, Sciaridae				
Sciarid species	R?	+	+	–
Diptera, Syrphidae				
Cheilosia sp.	R	+	–	–

Table 1 *(continued)*

Biotic agent	Part attacked	*C. maculosa*	*C. diffusa*	Other *Centaurea* spp.
Diptera, Trypetidae				
Acanthiophilus helianthi Rossi*	F/Se	+	+	○
Chaetorellia nr. *hexachaeta* Loew.	F/Se	+	+	?
C. jaceae R.D.	F/Se	+	−	○
Chaetostomella onotrophes Loew.*	F/Se	+	−	○
Terellia virens Loew.*	F/Se	(+)	(+)	−
Urophora affinis Frfld.	F/Se	+	+	○
U. quadrifasciata Meigen	F/Se	+	+	○
U. algira Macqu.	F/Se	+	+	○
Hymenoptera, Cynipidae				
Isolcus nr. *jaceae* (Schenk.)	Se	+	+	○
Phanacis centaureae (Foerst.)	Sh	+	+	○
Coleoptera, Curculionidae				
Apion onopordi Kirby	Sh/R	+	+	○
A. orientale Gersch.	Sh/R	+	−	○
A. penetrans Germar	Sh/R	+	+	○
Cleonus piger Scop.*	R	+	+	○
Cyphocleonus achates Fab.	R	+	+	○
C. tigrinus Panzer*	R	+?	+?	○
Larinus australis Cap.	F	+	−	○
L. minutus Gyll.	F	−	+	−
L. obtusus Gyll.	F	+	−	○
L. sturnus Schaller*	F	+	−	○
Coleoptera, Buprestidae				
Sphenoptera jugoslavica Ob.	R	−	+	?+
Coleoptera, Chrysomelidae				
Arima marginata F.*	L	−	L	+
Cassida pannonica Suff.	L	+	−	−
C. vibex L.(?)	L	−	+	○
Chrysomela fuliginosa Ol.*	L	+	−	○
Sphaeroderma rubidum (Graells.)*	L	−	+	+
Coleoptera, Anobiidae				
Lasioderma redtenbacheri Bach.	Se	+	+	○
Coleoptera, Mordellidae				
Mordelistena sp.	Sh	−	+	−

○, literature record; +, survey record; −, not recorded; *, known to attack other Cynareae.

Plant part attacked: cB, central bud of rosette; F, flower; L, leaf; R, root collar and root; Se, seed; Sh, shoot.

Human activities have been responsible for this rapid loss in number of suitable knapweed sites. The main culprits have been the direct application of herbicides as well as herbicide drift from nearby intensive cultivation, as well as burning and cutting along roadsides, embankments and other such wasteland. In addition, new residential and industrial construction destroyed many former spotted knapweed sites in the Rhine Valley and in eastern Austria, and the transformation of uncultivated, unimproved pastureland into vineyards, as now occurs in Bulgaria and mainly Romania, has taken a toll of prime diffuse knapweed areas. Consequently surveys for additional control agents become increasingly more difficult and require a higher investment than previously. Under these circumstances, the demand to concentrate field surveys on 'optimal' survey areas cannot any longer be fulfilled. Surveys have to be made wherever knapweed can still be found.

Biotic Agents Associated with Diffuse and Spotted Knapweed in Europe

According to a literature review and information obtained from collections and specialists in Europe the two knapweeds are attacked by 53 species of biotic agents. These include 2 species of rust, 2 mites and 49 insects (Table 1). Fourteen of the insect species recorded attack other Compositae in addition to *Centaurea* spp. During field surveys by the European Station, CIBC, and the USDA Biological Control of Weeds Laboratory, Rome, 2 fungal, 2 mite and 34 insect species were found on spotted knapweed, and 2 fungal, 1 mite and 34 insect species on diffuse knapweed.

Table 2. Exploitation of specific food niches on diffuse and spotted knapweed by biotic agents[1] in Europe.

	Flowers seeds	Leaves	Shoots	Root collar root	Total	%
Centaurea maculosa						
Lepidoptera	7	—	2	5	14	24.6
Diptera	8	1	1	4	14	24.6
Hymenoptera	1	—	1	—	2	3.5
Coleoptera	4	2	3	6	15	26.3
Others	1	6	3	2	12	21.0
Total	21	9	10	17	57	
%	36.8	15.8	17.6	29.8		100.0
Centaurea diffusa						
Lepidoptera	4	2	1	3	10	23.8
Diptera	7	—	1	3	11	26.2
Hymenoptera	1	—	1	—	2	4.8
Coleoptera	1	3	3	6	13	31.0
Others	—	4	1	1	6	14.2
Total	13	9	7	13	42	
%	31.0	21.4	16.6	31.0		100.0

[1]Agents occupying several niches are counted for each niche they occupy.

As a result of the scattered occurrence of the two knapweeds and their restricted abundance in most areas, well-developed complexes of phytophagous arthropods were seldom encountered during field surveys. The more specific agents, those species restricted to the target weeds and a few closely related *Centaurea* spp., were often absent from suitable sites within their general distribution area. For example, an analysis of the complex of root-feeding insects (12 species) at 37 localities revealed that, with the exception of *Apion* spp. (Coleoptera: Apionidae), none of the species occurred in more than a third of these localities, and that the rate of interspecific association was generally low (Müller, pers. comm.). In general, most of the host-specific arthropods were found in low number at the majority of the sites surveyed, and their occurrence over a longer period of time was quite sporadic. In these circumstances it is very difficult, if not impossible, for the survey entomologist to collect data on the impact of individual species on host plant distribution and abundance as proposed by Wapshere (1974*b*).

However, a large body of information has been collected on the time and mode of attack, as well as on the stress exerted on the attacked host plants, for most of the arthropod species closely associated with the two target knapweed species. Although no statistical analysis could be made on the impact of the arthropod community on the population dynamics of diffuse and spotted knapweed in their native range, these organisms undoubtedly exert a high consumer pressure; neither diffuse nor spotted knapweed are considered to be noxious weeds in Europe.

The Selection of Apparently Safe and Effective Biological Control Agents

It is generally recognized by biological control workers that the determination of the potential effectiveness of control agents is a very complex problem which is far from solved. Therefore the question of whether a control agent selected for host specificity screening will finally become an 'effective' control agent (i.e. will become established and affect the density and abundance of the target weed) cannot be answered in advance. However, certain guidelines have been developed during the past 20 years (e.g. Goeden 1983, Harris 1973, and Wapshere 1974*b*), which help the survey entomologist in selecting the most promising potential control agents from the complex of biotic agents encountered during field surveys. A suitable control agent should attack the target weed at an early stage or a critical time of its life cycle, for example when the plant is subjected to environmental stress. The kind of control agents needed for the successful biological control of a target weed are mainly determined by plant type (i.e. annual, biennial, or perennial), its mode of propagation and distribution, and the environment(s) where it occurs at noxious densities.

Diffuse knapweed is typically a biennial; spotted knapweed a short-lived perennial. Both species can overwinter as seeds or rosettes. Another common characteristic is their prolific seed production (up to 40 000/m^2 at high density) (Watson and Renney 1974) and their allelopathic properties (Fletcher and Renney 1963). The environmental conditions of Canadian infestations are discussed by Harris and Cranston (1979). Diffuse knapweed tends to dominate native vegetation in steppic grassland on Brown Chernozem and Brunisol in areas with an arid period in the summer. Spotted knapweed is most aggressive in the forest steppe zone on Dark Brown Chernozem. It does not compete with vigorously growing grass in moist sites nor with diffuse knapweed in steppic grassland.

To retard and possibly prevent the further spread of diffuse and spotted knapweed in North America and to reduce knapweed density in infestations, control agents feeding on flower heads and rosettes are of prime interest for biological control. Moreover, agents selected for introduction into North America have to be adapted to a steppic climate and their hibernating stages have to be able to survive the cold North American winters.

The Exploitation of Specific Food Niches by Arthropods Associated with Diffuse and Spotted Knapweed in Europe

Although the centre of the diffuse knapweed range and the eastern part of the spotted knapweed range, southern USSR, could not be surveyed, a good selection of potential biological control agents was found in the western part of the native distribution of the two knapweeds (Table 1). The exploitation of specific food niches on diffuse and spotted knapweed by specialized arthropods is shown in Table 2. The majority of species found feed on flower heads and rosettes, where they attack central buds and roots, respectively.

The general field surveys for potential control agents of diffuse and spotted knapweed and related species was terminated in 1971. By this time the life history of 12 species had been studied and the host range of 10 species had been screened. Based on the information accumulated, a list of potential control agents was established (Schroeder 1977) and updated later on (Table 3).

Table 3. List of potential biological control agents for diffuse and spotted knapweed and their host plant associations in Europe.

Agents attacking	*C. diffusa*	*C. maculosa*	*C. arenaria*	*C. vallesiaca*
Flowers and seeds				
Metzneria diffusella Englert	+	—	—	—
M. paucipunctella Zeller	—	+	—	+
Stenodes straminea (Haw.)	—	+	—	+
Urophora affinis Frfld.	+	+	—	—
U. quadrifasciata Meigen	+	+	—	—
Isolcus sp. nr. *jaceae* (Schenk.)	+	+	?	—
Shoots and leaves				
Puccinia centaureae DC.	+	+	—	—
P. jaceae Otth.	+	+	—	—
Aceria centaureae (Nal.)	+	+	—	+
Macrosiphoniella stägeri HRL.	—	+	—	+
Cassida pannonica Suff.	+	—	—	—
Phanacis centaureae (Foerst.)	+	+	—	—
Rosettes and roots				
Agapeta zoegana (L.)	—	+	+	—
Pelochrista medullana Stgr.	+	+	—	—
Stenodes meridiana Stgr.	—	—	+	—
S. straminea (Haw.)	—	+	—	+
Pterolonche inspersa Stgr.	+	+	—	—
Cheilosia sp.	—	+	—	—
Pegohylemyia centaureae Hennig	+	+	—	+
Cyphocleonus achates Fab.	+	+	—	—
Cleonus piger Scop.	+	+	—	—
Apion onopordi Kirby	+	+	—	+
A. penetrans Germar	+	+	+	—
Sphenoptera jugoslavica Ob.	+	—	—	—

The Selection of Potential Control Agents for Detailed Life-History Studies and Host Specificity Screening

Flowers and Seed-heads

In view of the prolific seed production of diffuse and spotted knapweed, the species attacking flowers and seeds were investigated first. Between 1967 and 1971, three of the six potential candidate species were studied and screened: *Urophora affinis* Frauenfeld, *U. quadrifasciata* Meigen (Diptera: Tephritidae), and *Metzneria paucipunctella* Zeller (Lepidoptera: Gelechiidae). They were found to be safe for release and were first released between 1970 and 1973 in British Columbia. After their establishment in British Columbia (Harris 1980*a*), releases of the two seed-head gall flies in the western United States followed between 1973 and 1980 (Maddox 1982). The flies have spread through most of the North American knapweed region, although dispersion of *U. affinis* is slower than that of *U. quadrifasciata*. Harris (1980*b*) reports a combined density related to the size of the knapweed flower head. The effect of the flies at the diffuse knapweed release site in British Columbia has resulted in a reduction of seed production from 30 000–40 000/m^2 to 1500/m^2.

M. paucipunctella attacks the seed heads of spotted but not diffuse knapweed. In British Columbia it currently attacks a third to half of the seed heads in the release stand but an average 20% of the seeds survive in attacked heads. In Europe, the moth is in fact considerably more damaging in the heads of *C. vallesiaca* which are smaller than those of the tetraploid spotted knapweed. *M. paucipunctella* coexists in the same heads with *U. quadrifasciata* but destroys the galls of *U. affinis*. Unless it can be demonstrated that *M. paucipunctella* increases the seed destruction presently achieved by the two gall flies, its distribution throughout the spotted knapweed area does not seem warranted.

In view of the impact of the combined effect of *U. affinis* and *U. quadrifasciata* on seed production of diffuse and spotted knapweed and the adaptability of the two flies to the climatic conditions in the North American knapweed area, it was decided to refrain from introducing further seed-head insects. Another two species, *Metzneria diffusella* Englert and *Isolcus* nr. *jaceae* (Schenk.) (Hymenoptera: Cynipidae), could be screened in the future if the introduction of additional species should be required. The remaining species, *Stenodes straminea* (Haw.) (Lepidoptera: Cochylidae), is no longer considered a potential control agent because it developed on a wide range of species in the tribe Cynareae, including globe artichoke and safflower, during host range screening.

Shoots and Leaves

With the exception of *Puccinia jaceae* Otth. (Pucciniaceae) and *P. centaureae* DC. (Mortensen and Harris 1983), the agents attacking shoots and leaves of diffuse and spotted knapweed have been little studied.

During 1978–80, samples of rust-infected diffuse and spotted knapweed were collected in central and eastern Europe. The material consisted of both *P. jaceae* and *P. centaureae*, but was predominantly *P. jaceae* (Watson *et al.* 1981; Watson and Alkhoury 1981). Collections from diffuse and spotted knapweed are under study at Regina and McGill University. The diffuse knapweed strain studied at Regina causes heavy infection with the attacked leaves senescing after about two weeks under laboratory conditions. The rust forms a resistant type reaction on safflower in the laboratory. Safflower is probably not damaged under field conditions and will be tested in southern France in 1984. If it can be demonstrated that safflower is not affected by the two strains of *P. jaceae* under field conditions in Europe, their release in North America can be proposed.

The leaf-gall mite *Aceria centaureae* (Nal.) (Acarina: Eriophyidae) has been observed only twice during the surveys, once in spring 1970 on *C. vallesiaca* in the Swiss Valais, and once on *C. diffusa* near Lake Skutari in southern Yugoslavia where it caused severe damage on the rosettes and shoots. The capacity of gall mites to migrate is very limited. It will therefore be difficult or impossible for *A. centaureae* to colonize scattered stands of diffuse and spotted knapweed. This may be the main reason why the mite was not found during field surveys between 1981 and 1983.

Macrosiphoniella stägeri HRL. (Homoptera: Aphididae) attacks *C. vallesiaca* and *C. maculosa* var. *rhenana*. It has been recorded from Switzerland and Germany, and also from Bulgaria and Poland where it occurs in the Pannonic steppes and other xerothermous habitats. The colonies develop on the tips of developing flowering shoots and young leaves. Heavily attacked plants die in early summer. The aphid was not found during the 1983–84 surveys, and apparently appears only occasionally in the western part of its host plant range.

Cassida pannonica Suff. (Coleoptera: Chrysomelidae) was not found during CIBC surveys; however, according to the literature it should be restricted to diffuse and spotted knapweed in the steppic region of Europe. It would seem that *C. pannonica*, like *A. centaureae* and *M. stägeri*, will only be found if the surveys can be extended to southern USSR.

Phanacis centaureae (Foerst.) (Hymenoptera: Cynipidae) forms galls in the vascular part of the stem. It is exceedingly rare in the western part of the diffuse knapweed range where it has been found only once since 1961.

Rosettes and Roots

On the assumption that the combined effect of *U. affinis* and *U. quadrifasciata* alone will not result in a general reduction in plant density of diffuse and spotted knapweed in North America, the release of agents which attack the rosettes and roots of the two knapweeds is advised. During surveys in Europe 12 species of potential interest were discovered (Table 3).

Sphenoptera jugoslavica Ob. (Coleoptera: Buprestidae), a root miner indigenous to the Balkans, was the first to be studied and screened (Zwölfer 1976). The larvae mine the central part of rosette roots of *C. diffusa* leaving a cylinder of cortex undamaged, thus are able to coexist with species that feed on the outside of knapweed roots. In Europe, attacked rosettes continue to live, but often do not flower in the following year and are subject to a second attack by *S. jugoslavica* or other root-feeding species. *S. jugoslavica* was released at two sites in British Columbia in 1976 and became established at one site (Harris and Myers 1984). The effect of *S. jugoslavica* on seed production and density of *C. diffusa* in the release site is presently being studied.

The analysis of the effect of biological control agents of diffuse and spotted knapweed in Canada has clearly indicated that additional species have to be established to reduce knapweed density below the economic threshold. Therefore, a detailed investigation of the complex of rosette- and root-feeding insects associated with diffuse and spotted knapweed and two additional *Centaurea* species, *C. arenaria* Bieb. ex Willd. and *C. vallesiaca*, was carried out by CIBC between 1979 and 1983 at 37 localities. The majority of species (with the exception of *Apion* spp.) occurred at only 40% or less of the localities studied, most likely because of the scattered host plant distribution. In addition, the rate of attack by individual species was generally low, with higher rates of attack being attained only in areas where the host plants occurred more frequently and at higher density.

Information on the occurrence of individual species in the area surveyed is now available. *S. jugoslavica* and *Pterolonche inspersa* Stgr. (Lepidoptera: Gelechiidae) are constantly associated with *C. diffusa* in Macedonia, but are absent in the eastern *C. diffusa* range, e.g. in Dobrugea, Romania, where they are replaced by *Pelochrista medullana* Stgr. (Lepidoptera: Tortricidae) and *Cyphocleonus achates* Fab. (Coleoptera: Curculionidae). These two species are widely distributed from eastern Romania westwards to eastern Austria, but are absent in the western part of the European spotted knapweed range. *Agapeta zoegana* (L.) (Lepidoptera: Cochylidae) is found throughout the spotted knapweed range surveyed, and on *C. arenaria* in eastern Romania. However, it is rare in western central Europe; e.g. the Rhine Valley. With the exception of *Apion* spp. and *Cleonus piger* Scop. (Coleoptera: Curculionidae) which apparently occur throughout the survey area, the remaining species seem to be restricted to the western and central parts of the survey area. Specifically *S. straminea* and *P. centaureae* occur more frequently and at higher density in the western part of the survey area; e.g. the Swiss Valais on *C. vallesiaca*.

During 1979 and 1981 the life history and host range of four species, *P. inspersa*, *A. zoegana*, *P. medullana*, and *S. straminea*, were studied. The Mediterranean *P. inspersa* was investigated by staff of the USDA Rome Laboratory. During host specificity screening with 49 plant species it was found that it is restricted to *C. diffusa* and accepts North American ecotypes of this species. It has been introduced into quarantine for additional host specificity screening. It is expected that *P. inspersa* will be cleared for release. In 1983 a relatively high percentage of *C. maculosa* was found attacked by *P. inspersa* in northern Hungary. This is the first CIBC record of this moth from spotted knapweed. The development of Hungarian *P. inspersa* on diffuse and spotted knapweed is presently being studied.

A. zoegana and *P. medullana* have been studied and screened simultaneously by the CIBC European Station. Both species have been cleared for release in Canada and the United States, and first releases in Canada were made in 1982 and 1983. The feeding of 1–2 larvae of *A. zoegana* destroys the roots of small rosettes, and half-grown larvae apparently actively search for additional food if a root does not sustain the complete development. This is of special interest with a view to the high plant density in North America. Moreover, multiple attack by *A. zoegana* is quite frequent, attaining 60% on *C. maculosa* and 76% on *C. arenaria*. Thus the complete destruction of big rosette roots occurs quite frequently.

P. medullana predominantly attacks *C. diffusa*, but accepts *C. maculosa* in the absence of *C. diffusa*. In contrast to *A. zoegana*, only a single larva develops on each root. Nevertheless, tests at the CIBC European Station have demonstrated that feeding of a single larva causes serious damage. Over two-thirds of well-developed rosettes were killed in late autumn prior to the hibernation of *P. medullana*. Field and laboratory observations also demonstrated that the two moths coexist without deleterious effects on either species. It is expected that the two species of cochylid moths will have an important impact on knapweed density in North America after they have become firmly established.

S. straminea has two generations a year, the autumn generation attacking the central bud and root-collar of rosettes, the summer generation the flower heads. In spite of the damage inflicted by *S. straminea* to knapweed, it had to be rejected as a biological control agent because it could develop on a wide range of species in the tribe Cynareae, including globe artichoke and safflower.

Most of the remaining seven species have no or only limited interest for biological control. *Apion* spp., although widely distributed and common, cause apparently little stress and do not affect plant growth and seed production. *Pegohylemyia centaureae* Hennig (Diptera: Anthomyiidae) is a localized species that occasionally attains high population levels on *C. vallesiaca*. Although recorded to attack diffuse and spotted knapweed, it was not found on these species during CIBC surveys. Moreover, it was not possible to breed *P. centaureae* in captivity. The same is true for *Cheilosia* sp. (Diptera: Syrphidae), which was frequently encountered in eastern Austria and Hungary in 1979 and 1980, but the populations declined since, preventing the continuation of investigations started in 1980. *Stenodes meridiana* Stgr. was found feeding on the roots of *C. arenaria* in southeastern Romania in 1980 and 1981, but was not collected in 1984. *S. meridiana* remains of interest as a potential control agent and should be studied as soon as larvae are found.

An investigation of the biology and host range of *C. achates* and *C. piger* commenced at the CIBC European Station in 1984. According to CIBC observations, the host range of *C. achates* is restricted to diffuse and spotted knapweed. Its larvae mine the roots. The shoots of attacked plants are significantly shorter than those of healthy plants and have less flowers. *C. achates* occurred with seven other root-feeding species at various localities, but coexisted only with *Apion* spp. on the same root. Interspecific competition with other root-feeding species is apparently a rare event. If it can be demonstrated that the host range of *C. achates* is restricted to knapweed, it can be considered for release in North America.

Unlike *C. achates*, *C. piger* has a wide host range and is reported to attack globe artichoke (La Ferla 1939). It is suspected that host strains exist for *C. piger*, which is common on various thistles in Europe, but which is also consistently associated with diffuse and spotted knapweed. Therefore, ovariole development, oviposition preferences, and larval survival of *C. piger* from knapweed are being studied to determine if a narrowly specific host race can be found.

Host Specificity Determination of Biological Control Agents

Screening of potential host range (host specificity) is the most crucial operation in any biological control project. Because of the overriding importance of safety, the greatest care has to be taken in selecting the appropriate test plants and in designing tests to demonstrate the safety of potential control agents beyond reasonable doubt. General considerations of this problem are found in Harris and Zwölfer (1968), Zwölfer and Harris (1971) and Wapshere (1974*a*).

The number of plants which must be included in the screening programme depends mainly on: (*a*) the taxonomic position of the target weed — whether it belongs to an isolated family or a family with close relations; (*b*) the number of closely related cultivated plants, and wild plants, which should not be attacked; (*c*) the geographic and/or ecological isolation of the release area; and (*d*) whether or not the control organism belongs to a systematic group which is known to be restricted to a small group of closely related plants (genus, subtribe, tribe) (Schroeder 1983).

Diffuse and spotted knapweed belong to the tribe Cynareae of the Compositae with a relatively large number of species, native and introduced, in the release area. Moreover, two important crops, safflower and globe artichoke, and several ornamental species cultivated in North America belong to the tribe Cynareae. This has been taken into consideration during host specificity screening of biological control agents which have been released in North America. A total of 36 plant species were screened in case of *U. affinis*, *U. quadrifasciata*, and *M. paucipunctella*, and 46 with *S. jugoslavica*. Before permission for the release of *A. zoegana* and *P. medullana* was obtained in 1982, a total of 54 plant species had to be screened, and a similar or larger number will have to be screened with *P. inspersa* and *C. achates*, and other potential control agents.

One important reason for the increased number of test plant species is the concern that insects released against knapweed might attack rare and endangered native Cynareae, especially thistles. In addition, several species of native and alien ornamental plants were tested to avoid conflicts of interest. Although the concerns of environmentalists and amateur gardeners are appreciated and seriously considered by biological control workers, the danger has to be realized that otherwise safe and effective control agents might have to be rejected in the future because of these extended safety requirements.

In view of the great economic important of diffuse and spotted knapweed to the range industry in North America, the fact that both weeds tend to dominate and suppress native flora over large areas, and danger of further spread of the weeds (Harris and Cranston 1979), the justified concerns of environmentologists and amateur gardeners have to be carefully weighed and should be subject to further discussion. It should be borne in mind that diffuse and spotted knapweed apparently cannot be controlled or even contained by chemical or mechanical means, and that biological control seems to be the sole and economically most rewarding method to resolve this important weed problem. Demands for additional test plant species should therefore be carefully assessed and only accepted after all economic and ecological aspects have been considered. It may not be possible to resolve all conflicts of interest (Andres 1981), but with improved communication between all concerned parties it should be possible to resolve most of them.

Conclusions and Recommendations

(1) As illustrated by the example of spotted knapweed, the taxonomy of a target weed may not be well known at the time when foreign exploration is being started. In such cases taxonomic studies have to be carried out in the presumed native range and the area colonized to clarify the taxonomic status of the target weed and to locate the identical taxum(a) in the native distribution area. At the same time potential biological control agents should be collected from closely related taxa (subspecies or species) and their ability to attack and develop normally on the target weed should be tested.

(2) The generally accepted guideline that foreign exploration for safe and potentially effective control agents should start at optimal survey areas (i.e. where a climatically similar region geographically overlaps the area of greatest diversification of the genus or subgenus to which the target weed belongs) could not be followed for diffuse and spotted knapweed. The best suitable survey area in southern USSR is not accessible. Surveys for biological control agents had therefore to be carried out in the marginal western range of their native distribution, in areas climatically less similar to the release areas. Nevertheless, for diffuse and spotted knapweed, the results of the field surveys justify the decision to survey marginal areas if the potentially best survey areas are not accessible.

(3) The selection of apparently safe and effective biological control agents is a difficult task for all scientists involved in foreign exploration. It is especially difficult to find such agents if field surveys have to be carried out in marginal areas of the native distribution of the target weed(s), where the plant occurs in small scattered stands and a well-developed complex of phytophagous arthropods is seldom encountered. This applied for diffuse and spotted knapweed during field surveys in central and western Europe. It has therefore not been possible to determine the impact of individual arthropod species on the distribution and abundance of the two knapweeds. However, under consideration of the biology and ecology of diffuse and spotted knapweed in North America, it has been possible to select a number of potential biological control agents by means of direct field observations and laboratory investigations in Europe.

In Europe, suitable survey areas for biological control agents of introduced North American weeds are becoming more and more rare. With a view to guarantee the further supply of control agents it may become necessary to rent and manage suitable areas in the future.

(4) The overriding importance of safety in any biological weed control operation is generally acknowledged. However, the tendency to extend the safety demands beyond

certain limits may be detrimental to biological weed control; e.g. the demand that alien control agents should not attack any rare or endangered native plant species in the release area. In case of diffuse and spotted knapweed, an otherwise safe and effective control agent may be rejected because it feeds on a rare North American *Cirsium* species (Compositae) during host specificity testing.

In view of the great economic importance of knapweed and other introduced weed species, such as leafy spurge, to the North American range industry, demands for test plant species should be carefully assessed and only accepted after all economic and ecological aspects have been considered. It would seem to be unwise and detrimental if biological weed control would be paralyzed by overstressed and/or unjustified safety demands. With improved communication between all concerned parties it should be possible to resolve most conflicts of interest associated with biological weed control.

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