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OF THE TWENTY-FIRST CENTURY**

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Coeur d'Alene, Idaho

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**Edited by Lincoln Smith**

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## PAPERS

### General Session

#### **Knapweed Management: Another Decade of Change**

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#### **Abstract**

Knapweeds (*Centaurea* sp.) are a major threat to the ecology and economy of western states. Their ability to establish on a wide range of ecological sites and their impacts on structure and function of ecological systems continues to cause concern among scientists and land managers. Knapweed Symposiums held in 1977, 1984 and 1989 were successful in identifying needs and priorities, and promoting knapweed research, management, and public education and awareness programs in the western region.

The purpose of this paper is to review progress in knapweed management and changes in knapweed distribution during the past decade. Acreage infested, management strategies and philosophy, research, and public education and awareness will be reviewed for four major knapweed species: Spotted knapweed (*Centaurea maculosa* Lam), diffuse knapweed (*Centaurea diffusa*), Russian knapweed (*Centaurea [Acroptilon] repens*), and yellow starthistle (*Centaurea solstitialis*).

Key words: *Centaurea* spp., management, biocontrols

#### **Methods:**

##### **Data Collection**

In 1988 survey questionnaires on knapweed acreage and management were mailed to University Weed Extension Specialists or State Department of Agriculture weed specialists in Colorado, Washington, Oregon, Idaho, Montana, Utah, Wyoming, North Dakota, South Dakota, and British Columbia, and Alberta Canada. Similar questionnaires were mailed in 2000 to these states, and this survey was expanded to include California, Nevada, Arizona, New Mexico, Kansas, and Nebraska. Responses were received from 16 of the 17 states. Data were summarized from the questionnaire and compared to responses from 1988. For the purpose of this document, data from states and provinces are reported together and "provinces" are synonymous with "states". In addition to the questionnaire, internet sites were utilized to obtain information on knapweed distribution, and published research and extension articles were derived from a literature search.

#### **Results:**

##### **Knapweed Distribution**

The four knapweed species infest an estimated 32.3 million acres in 16 western states, with yellow starthistle infesting between 15 and 20 million acres in California alone (Table 1). Although yellow starthistle was reported in only 68% of states surveyed, the weed had the greatest spread rate during the past decade.

Table 1: Knapweed acreage reported by 14 states and 2 Canadian provinces.

| State/<br>Province | Acreage <sup>1</sup> |           |                  |           |                  |           |                    |            |
|--------------------|----------------------|-----------|------------------|-----------|------------------|-----------|--------------------|------------|
|                    | Spotted Knapweed     |           | Diffuse Knapweed |           | Russian Knapweed |           | Yellow Starthistle |            |
|                    | 1988                 | 2000      | 1988             | 2000      | 1988             | 2000      | 1988               | 2000       |
| Colorado           | 2,500                | 2,500     | 30,000           | 83,000    | 50,000           | 168,000   | 10                 | 100        |
| Idaho              | 2,293,000            | 2,300,000 | 1,450,000        | 1,800,000 | 890,000          | 425,000   | 1,130,000          | 800,000    |
| Montana            | 4,721,060            | 3,818,450 | 10,349           | 27,523    | 47,893           | 64,456    | 1                  | 1          |
| N. Dakota          | 0                    | 1,160     | 0                | 30        | 250              | 436       | 0                  | 400        |
| Oregon             | 3,000                | 784,000   | 1,200,000        | 989,000   | 15,000           | 85,000    | 10,000             | 950,000    |
| S. Dakota          | 2,500                | 1,898     | 1,000            | 200       | 3,045            | 2,717     | 0                  | 0          |
| Utah               | 500                  | 2,000     | 25               | 1,300     | 150,000          | 60,000    | 100                | 2,200      |
| Washington         | 29,070               | 500,000   | 427,800          | 500,000   | 8,050            | 500,000   | 133,805            | 1,000,000  |
| Wyoming            | 100                  | 15,000    | 5,000            | 4,000     | 200,000          | 160,000   | 0                  | 0          |
| Alberta            | 0                    | scattered | 0                | scattered | 20               | scattered | 0                  | 0          |
| British Col.       |                      | 50,000    |                  | 75,000    |                  | 450       |                    | 0          |
| Arizona            |                      | 1,800     |                  | 1,800     |                  | 5,500     |                    | 3,000      |
| California         |                      | 5         |                  | 5         |                  | 150       |                    | 17,000,000 |
| Kansas             |                      | 0         |                  | 0         |                  | 5         |                    | 0          |
| Nevada             |                      | 5000      |                  | 500       |                  | 75,000    |                    | 5,000      |
| New Mexico         |                      | 500       |                  | 200       |                  | 15,000    |                    | 500        |
| Total              |                      | 7,482,313 |                  | 3,482,558 |                  | 1,561,714 |                    | 19,761,201 |

<sup>1</sup> 1 acre = 0.40 hectares

Spotted and diffuse knapweed were reported in all states surveyed except Kansas, with the largest infestations reported in Montana and Idaho. Diffuse knapweed infested the greatest acreage in Idaho and Oregon. Russian knapweed was the only species reported in all states included in the survey, but infested only 1.5 million acres.

Knapweed acreage figures were available for both 1988 and 2000 for eleven western states. Results from these 11 states showed an increase in spotted, diffuse, and Russian knapweed, and yellow starthistle of 6.5, 12, 7, and 116 percent, respectively. Improved inventory methods are responsible for a decrease of about 1 million acres of spotted knapweed in Montana during the past decade.

Results from the survey indicated that, although inventory methods improved and more inventories were conducted during the past decade, accuracy remained relatively low. More than half of the states reported that their acreage estimates were less than 50% accurate, while 31% reported between 51 to 75% accuracy. Only 2 states reported that knapweed acreage estimates were 75 to 100% accurate.

## Management Programs

### *Biological Control and Grazing Animals*

Collection, screening, and release of biocontrol agents continues to be a major component of knapweed management on non-crop sites in the western region. From 1988 to 2000 the number of agents released and established on spotted and diffuse knapweed



increased more than 2-fold. The number of insects established for management of yellow starthistle increased three-fold from 1988 to 2000 (Table 2). Although the nematode remains the only agent currently established on Russian knapweed, insects are currently being screened for release on the weed (Story, pers. comm.)

The past decade has seen a substantial improvement in coordination and collaboration between state and federal research scientists, extension specialists, and weed managers. Funding for foreign screening and collection of insects through CABI was substantially increased for spotted and diffuse knapweed mainly as a result of Montana's Noxious Weed Trust Fund, the Canadian government, and USDA. In addition, mass rearing, collection, and redistribution techniques for biocontrol agents have been developed and promoted throughout the western region.

Research continues on use of the fungus *Sclerotinia sclerotiorum*. Although the host range of the fungus was reduced, its practical application as a management tool for knapweeds has not been realized. There has been no work completed on the parasitic fungus, *Alternaria alternata* that was isolated on spotted knapweed in 1988.

The use of grazing animals for management of knapweeds has continued to increase during the past decade. Most states report limited use of sheep and/or goats for management of knapweed infestations. Research has been conducted regarding physical effects of clipping on spotted knapweed, and effect of controlled, repeated sheep grazing as a management tool. Intensive grazing with a large number of animals for short duration has been shown to reduce plant height, canopy size, and seed production of yellow starthistle. Manipulating the type of livestock, time of use, and combining grazing with other management techniques such as herbicides or biocontrol agents may prove to be a valuable knapweed management tool during the next decade.

### ***Chemical Control***

Herbicides continued to be an important component of knapweed management programs since 1989. Imazapic (Plateau) is a new herbicide marketed since 1989 that has activity on knapweeds. Selective broadleaf herbicides that are effective against the knapweeds and starthistle include picloram (Tordon 22K), clopyralid (Transline, Curtail, Redeem), 2,4-D, and dicamba (Banvel). Research regarding optimum time of application and application rate has continued during the past decade. There was a significant change during the past decade from herbicide-only research to integrated projects combining herbicides with other management techniques.

One of the greatest needs identified during the 1989 Knapweed Symposium was to improve environmental monitoring prior to management programs involving herbicides. Since that time, federal agencies have developed Environmental Impact Statements and Environmental Assessments that include evaluation of soil, vegetation, surface water and groundwater resources and potential impacts from herbicides. The Montana Department of Agriculture requires Environmental Assessments prior to providing state cost-share funds for knapweed management. Increased awareness and public education on herbicide movement and protecting groundwater resources by private and public entities has improved herbicide use on private lands.

Table 2: Status of biocontrol agents on diffuse, spotted, and Russian knapweed and yellow starthistle (Coombs, Ali, and Cranston pers. comm.).

| Plant                          | Biological control agent        | Distribution Status of Biocontrol Agents by State <sup>1,2</sup> |    |    |    |    |    |    |    |    |    |    |     |    |
|--------------------------------|---------------------------------|--|----|----|----|----|----|----|----|----|----|----|-----|----|
|                                |                                 | AZ   | CA | CO | ID | MT | NV | NM | OR | UT | WA | WY | ALB | BC |
| Diffuse knapweed               | <i>Agapeta zoegana</i>          | F  | —  | L  | —  | L  | F  | —  | U  | L  | —  | L  | —   | L  |
|                                | <i>Bangasternus fausti</i>      | —  | L  | F  | U  | U  | —  | —  | L  | U  | F  | F  | —   | —  |
|                                | <i>Cyphocleonus achates</i>     | —  | —  | L  | —  | L  | —  | —  | L  | L  | L  | L  | U   | W  |
|                                | <i>Larinus minutus</i>          | —  | L  | L  | —  | L  | L  | —  | L  | L  | L  | W  | —   | L  |
|                                | <i>Metzneria paucipunctella</i> | —  | —  | —  | —  | L  | —  | —  | L  | —  | L  | —  | —   | L  |
|                                | <i>Pterolonche inspersa</i>     | —  | —  | F  | U  | F  | —  | —  | L  | —  | F  | —  | —   | F  |
|                                | <i>Sphenoptera jugoslavica</i>  | F  | W  | L  | W  | L  | L  | —  | W  | L  | W  | L  | —   | W  |
|                                | <i>Urophora affinis</i>         | F  | W  | W  | W  | W  | F  | —  | W  | L  | W  | W  | L   | W  |
|                                | <i>Urophora quadrifasciata</i>  | F  | L  | W  | W  | W  | F  | —  | W  | W  | W  | W  | L   | W  |
| Spotted knapweed               | <i>Agapeta zoegana</i>          | —  | L  | L  | L  | L  | F  | —  | L  | L  | L  | L  | —   | W  |
|                                | <i>Bangasternus fausti</i>      | —  | —  | F  | U  | U  | —  | —  | L  | —  | —  | F  | —   | —  |
|                                | <i>Chaetorellia acrolophi</i>   | —  | —  | L  | —  | U  | —  | —  | L  | —  | L  | L  | —   | L  |
|                                | <i>Cyphocleonus achates</i>     | —  | L  | W  | U  | L  | F  | L  | L  | L  | L  | L  | U   | W  |
|                                | <i>Larinus minutus</i>          | —  | W  | L  | L  | L  | L  | —  | L  | L  | L  | W  | —   | L  |
|                                | <i>Larinus obtusus</i>          | —  | —  | U  | U  | L  | —  | —  | L  | —  | L  | L  | —   | L  |
|                                | <i>Metzneria paucipunctella</i> | —  | —  | L  | W  | L  | —  | —  | W  | —  | W  | F  | —   | W  |
|                                | <i>Pelochrista medullana</i>    | —  | —  | —  | —  | L  | —  | —  | U  | —  | —  | —  | —   | F  |
|                                | <i>Pterolonche inspersa</i>     | —  | —  | F  | —  | U  | —  | —  | U  | —  | —  | —  | —   | F  |
|                                | <i>Sphenoptera jugoslavica</i>  | —  | —  | U  | —  | L  | U  | —  | L  | U  | —  | U  | —   | W  |
|                                | <i>Terellia virens</i>          | —  | L  | F  | —  | U  | —  | —  | L  | —  | U  | U  | —   | L  |
|                                | <i>Urophora affinis</i>         | —  | W  | W  | W  | W  | L  | —  | W  | L  | W  | W  | L   | W  |
| <i>Urophora quadrifasciata</i> | —                               | W  | W  | W  | W  | L  | —  | W  | W  | W  | W  | L  | W   |    |
| Russian knapweed               | <i>Subanguina picridis</i>      | —  | —  | L  | —  | L  | —  | L  | L  | L  | L  | L  | L   |    |
| Yellow starthistle             | <i>Bangasternus orientalis</i>  | F  | W  | —  | W  | —  | —  | —  | W  | L  | W  | —  | —   | —  |
|                                | <i>Chaetorellia australis</i>   | —  | L  | —  | W  | —  | —  | —  | W  | —  | W  | —  | —   | —  |
|                                | <i>Eustenopus villosus</i>      | L  | W  | —  | W  | —  | —  | —  | W  | U  | W  | —  | —   | —  |
|                                | <i>Larinus curtus</i>           | —  | L  | —  | W  | —  | —  | —  | W  | —  | L  | —  | —   | —  |
|                                | <i>Urophora jaculata</i>        | —  | F  | —  | F  | —  | —  | —  | —  | —  | F  | —  | —   | —  |
|                                | <i>Urophora sirunaseva</i>      | F  | W  | —  | W  | —  | —  | —  | W  | —  | L  | —  | —   | —  |

<sup>1</sup> State or province: AZ = Arizona, CA = California, CO = Colorado, ID = Idaho, MT = Montana, NV = Nevada, NM = New Mexico, OR = Oregon, UT = Utah, WA = Washington, WY = Wyoming, ALB = Alberta, BC = British Columbia

<sup>2</sup> Distribution: W = widespread in host range, L = established at limited sites, F = failed, U = unknown

The impact of herbicides on non-target forbs has been completed and published (Rice and Toney 1996, 1998; Rice et al. 1992, 1997). Results of the research indicate that 2 years after herbicide application on knapweed dominated sites, plant communities were not converted to grass monocultures with either Tordon 22K at 1 pint per acre, Curtail at 2 quarts

per acre, or Transline at 2/3 pints per acre. No large declines in plant diversity were caused by herbicide treatments, and small depressions were transitory.

### **Public Awareness**

Public awareness programs that target the knapweeds have been implemented in many states. Bounty programs, field tours, extension bulletins, newspaper articles, radio and television public service announcements, youth programs, and bumper stickers continue to be utilized to increase public awareness.

The survey asked respondents to estimate percent of agricultural producers and non-agricultural public that perceived knapweeds as a serious environmental threat. Eighty-seven percent of states reported less than 25% of the non-agricultural public perceived knapweeds as a serious environmental threat. Results were similar for agricultural producers. Respondents were also asked to estimate percent of agricultural producers that perceived knapweeds as a threat to agricultural production. Only twenty percent of states reported that more than 50% of agricultural producers perceived knapweeds as a serious threat to agricultural production. Public awareness was greatest for spotted knapweed. These results indicate that additional educational and awareness programs are needed for all knapweeds.

### **Cooperative Weed Management Areas:**

The concept of cooperative weed management areas, where groups of individuals and/or agencies manage knapweed in partnerships or other cooperative program was first discussed at the 1984 Knapweed Symposium. Since that time, the benefits of cooperative control efforts, and state and federal cost-share programs have promoted implementation of these projects. From 1988 to 2000, the average number of cooperative weed management areas in each state increased from 7 to 39. The number of states providing cost-share for knapweed management programs increased from 3 in 1988 to 7 in 2000. The most extensive cost-share programs are in California, Oregon, and Montana.

### **Research and Extension Programs**

Research scientists and extension specialists continue to recognize management of the knapweeds as a priority in the western region. The percent of time spent on knapweed research/extension programs increased from an average 14.9% in 1978 to 24.2% in 1988 and 34% in 2000. The number of full-time state employees involved with knapweed also increased from an average per state of 1.9 FTE in 1988 to 4.8 FTE in 2000.

A review of published literature from 1989 through 1998 indicates continuing emphasis and funding availability for knapweed research. Total number of citations on the knapweeds increased 34% between 1989 and 1993, and 21% from 1993 to 1998 (Figure 1). The greatest change in types of publications was a 37% decline in herbicide-only research, a 275% increase in integrated management techniques, and 112% increase in publications on biology and ecology of the knapweeds.

### **Legislation**

Legislation continues to be enacted to establish cost-share funds, restrict movement of knapweed, and strengthen weed control laws. Thirty-one percent of states had legislation that prohibited sale or transport of hay infested with spotted, diffuse, or Russian knapweed seed. Only 25% of states had similar legislation on yellow starthistle. Voluntary weed seed free forage programs are on-going in 5 states.

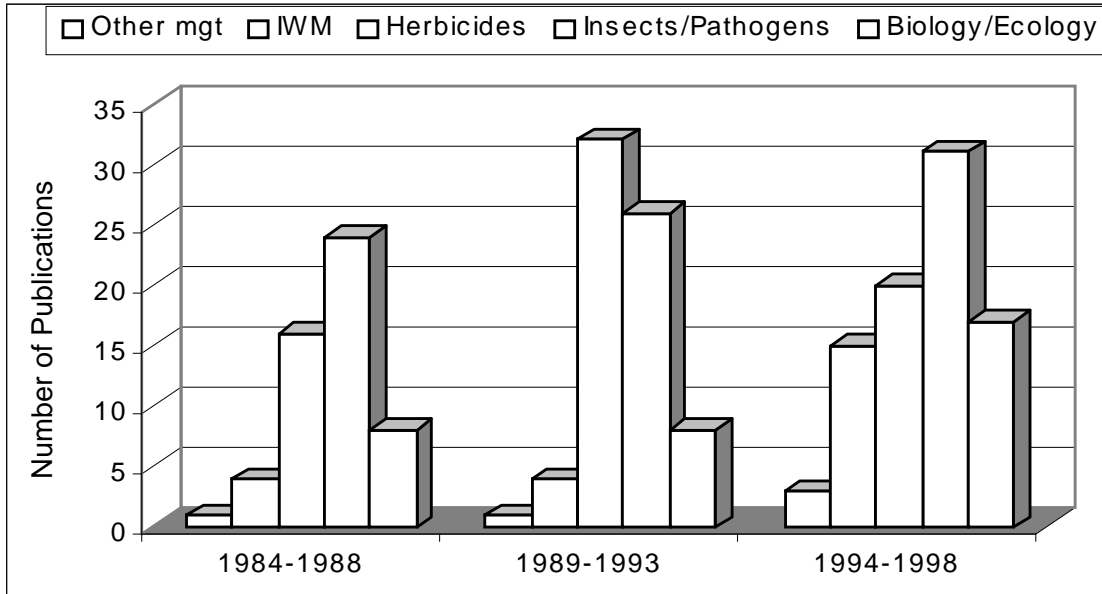


Figure 1: Published research reports on spotted, diffuse, and Russian knapweed and yellow starthistle from 1984 through 1998. Information compiled from Chemical Abstracts, Agricola, CAB, Science Citation Index, Biological Abstracts, and Crop Protection.

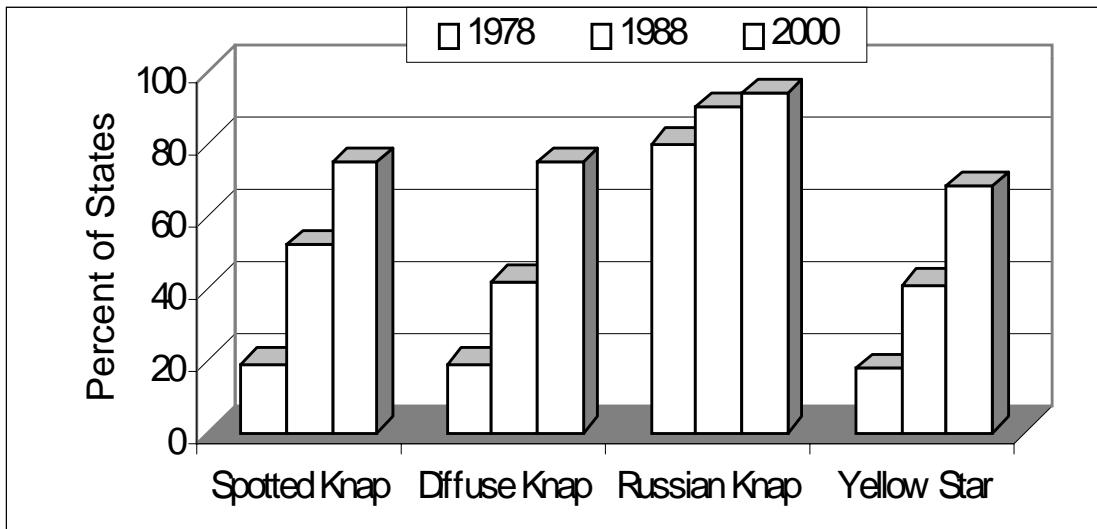


Figure 2: Percent of states with legislation prohibiting sale of certified seed contaminated with knapweed seed

All states in the survey reported legislation that prohibited sale of certified seed contaminated with at least one of the knapweeds (Figure 2). These results indicate substantial progress during the last 20 years in developing legislation to support viable weed management efforts.

## Summary

Significant progress in knapweed management has continued to occur within the western region during the past decade. The number of biocontrol agents established on the knapweeds and yellow starthistle have more than doubled. Integrating management techniques such as herbicides with biocontrol agents and grazing animals, fertilization, and restoration efforts have expanded. Legislation, public education programs, research, and expanding cooperative weed management programs has slowed spread of the knapweeds. However, expanding and improving current programs is needed to adequately address the magnitude of knapweed infestations. The key to long-term management is gaining a better understanding of the biology and ecology of these species, improving restoration techniques, expanding inventories to more accurately track infestations, increasing public awareness and education, and implementing cooperative integrated weed management programs.

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## **Knapweed Eradication Program in Alberta**

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### **Abstract**

Diffuse and spotted knapweed (*Centaurea diffusa* and *C. maculosa*) are major problem weeds on the rangelands of the north-western United States and western Provinces of Canada. These weeds form solid stands which reduce forage production and thereby reduce carrying capacity. Diffuse and spotted knapweed were first found in Alberta in 1974. To protect the 2.5 million acres of susceptible rangeland, an eradication program was undertaken in 1975. A search and destroy program was launched and by 1985 infestation levels were reduced to scattered plants. Program awareness and eradication measures have been maintained and Alberta is virtually knapweed-free.

Key words: diffuse knapweed, spotted knapweed

### **Background**

Diffuse and spotted knapweed (*Centaurea diffusa* and *C. maculosa*) are serious problem weeds on the rangelands of the North-Western U.S. and Western Provinces of Canada. These weeds reduce forage production, decrease carrying capacity and form solid stands and virtually eliminate all other vegetation.

Diffuse and spotted knapweed were first found in Alberta in 1974 along the railroad tracks in the Southern part of the Province. It was quite obvious from the experience in British Columbia and Montana that, if left alone, these plants could develop into a serious weed problem and infest up to 2.5 million acres of Alberta's rangeland.

In 1975, a survey was conducted to determine the level of infestation in the Province. The survey revealed a total of 370 acres, spread along 200 miles of transportation routes and a few industrial sites. The largest infestation was 2 acres in size while most of the infestations were small patches or isolated plants.

From 1975 through 1979 a search and destroy program on knapweed was undertaken by the Provincial Department of Agriculture. Although all infestations were treated, follow up measures were inadequate because of limited resources and the large area to cover by the few staff. More importantly, diffuse and spotted knapweed were not designated as weeds under the existing weed legislation and therefore enforcement measures upon the landowners could not be taken. By the end of 1979, knapweed infestations were reduced but the program itself was not very successful because control measures were solely upon the Provincial government.

In January 1980, Alberta's new weed control legislation came into force. One of the major changes was the classification of weeds into three categories: Restricted, Noxious and Nuisance. The important aspect of this categorization is that enforcement and eradication measures are mandatory for restricted weeds whilst with Noxious weeds the enforcement can be discretionary. Nuisance weeds are weeds which are in high numbers and enforcement is not practical except for activities that involve spread.

### **Program Objective**

The potential threat posed by diffuse and spotted knapweed to Alberta's rangelands makes it imperative to eradicate these weeds while it is still economically and physically feasible. The objectives of the Knapweed Eradication Program are:

- (1) To seek out and destroy every infestation of diffuse or spotted knapweed.
- (2) To make Alberta free of diffuse and spotted knapweed.

### **Methods**

The eradication program is designed to be a cooperative effort between the Provincial Government, Municipal or Local government and the landowner. The landowner being any person or organization who owns the land or has control of that land. Roles were assigned and communicated to each party to ensure that all phases of the program are carried out methodically and in a timely manner.

The Provincial government, Alberta Agriculture, Food and Rural Development, provides overall program coordination and leadership. It prepares and distributes awareness materials, publicity, conducts local extension programs, maintains an infestation inventory, alerts local weed inspectors to the presence of knapweed and monitors the eradication program. It also maintains contact with out-of-province officials and agencies to solicit their assistance and cooperation in the eradication efforts.

The Municipal Governments or Agricultural Service Boards are responsible for the enforcement of eradication measures within their own jurisdiction. They enforce the weed law and ensure that clean-up measures are undertaken in a timely manner. They also keep record of local infestations and alert neighboring landowners of these infestation and request that they search their land.

The landowner has the ultimate responsibility to eradicate knapweed from their land. By law, it is the responsibility of the landowner to control weeds on his land or land under his control. However, assistance through funding or partial subsidy in chemicals or equipment is made available on a per need basis. It is never intended to take the eradication responsibility away from the landowner.

The above roles assigned are in keeping with the normal responsibilities of each person or agency. All advertising and public information are related only to identification and reasons for the eradication programs with the emphasis on the threat that these knapweeds pose to over 2 million acres of Alberta's rangeland.

### **Results**

In 1975 the total infestation of diffuse and spotted knapweed was 370 acres. Most infestations were small, ranging from a few plants to 2 acres. The majority of these infestations were located along the railway tracks from the British Columbia border in the west through the Crowsnest Pass to the Saskatchewan border in the east, a distance of about 200 miles. There were also small, isolated infestations north and south of this transportation corridor.

The Eradication Program has been well-conducted by all local agencies. All known infestations have been subjected to eradication measures and retreated as necessary upon re-inspection. Measures taken are both chemical and mechanical.

The use of picloram herbicide has been most effective. Its residual characteristic has resulted in up to a 3-year control from a single application. In gravelly soils or soils of high carbon content, however, control was limited to one or two years. The Crowsnest Pass where

there are a number of knapweed sites in an area of coal deposits, annual applications of picloram is necessary.

In areas where picloram cannot be used, 2,4-D, dicamba or glyphosate is used. Dicamba and 2,4-D are only effective in the early rosette stage.

Mechanical methods include hand pulling and mowing. Hand pulling is widely used close to water bodies where chemicals cannot be applied or where there are isolated plants. Mowing is done mainly in urban areas but has been only effective in preventing seed set.

By 1985 infestation levels were reduced to isolated plants and by 1988 many sites have been eradicated as determined by no regrowth for at least 5 years. In 1994 the isolated plants level has been maintained. To date, most sites have been eradicated and annual inspections continue to ensure that reinfestation does not occur. Results are summarized in Table 1.

Table 1. Knapweed Infestation Levels

| <b>Year</b> | <b>Infestation Level</b>                  |
|-------------|---|
| 1975        | 370 acres                                 |
| 1985        | isolated plants                           |
| 1988        | isolated plants, many sites eradicated    |
| 1994        | isolated plants, several sites eradicated |
| 2000        | isolated plants, most sites eradicated    |

### **Ongoing Activities**

The Knapweed Eradication Program has been very successful through the active participation of all stakeholder groups. Due to seed dormancy and seed bank reserves eradication will be only achieved by ongoing monitoring of all known infestations and retreating where necessary. The entire area surrounding each infestation has to be inspected regularly to ensure that infestations are confined to the presently known areas.

Program awareness has to be maintained and extended to society as a whole. The general public has to be made aware of the potential threat of these weeds and ways to prevent their spread.

The greatest challenge will be to prevent the entry of knapweed via the international and provincial borders. Since over 65% of the known infestations are along railways and highways, it is reasonable to speculate that vehicular activities can be a major source of reinfestation. In this respect, we request the cooperation of our neighbors, British Columbia and Montana, to assist us by maintaining knapweed-free buffer zones close to our borders.

The importation of knapweed-infested hay is another area of concern. Border inspections and promoting the purchase of knapweed-free hay may reduce the entry of knapweed by hay movement.

### **Summary**

If the interest and activities of government authorities and landowners in the Knapweed Eradication Program are indicators, knapweed will not survive in the Province of Alberta. Alberta ranchers can anticipate that their ranges will not be ravaged by knapweed.



## **Spotted and Diffuse Knapweed**

### **Status of Biological Control Efforts against Spotted and Diffuse Knapweed**

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#### **Abstract**

Spotted knapweed, *Centaurea maculosa* Lamarck, and diffuse knapweed, *Centaurea diffusa* Lamarck, are Eurasian plants that have become weed problems on western rangelands. Efforts to biologically control spotted and diffuse knapweed have been underway in the United States since 1973. Thirteen Eurasian natural enemies (all insects), extensively tested to prove that they will not attack non-target plants, have been introduced for biological control of the two knapweeds in North America. Several of the insect species are demonstrating some impact against one or both of the two knapweed species. Status of the 13 insects and the progress of the overall biocontrol effort are reported.

Key words: *Centaurea maculosa*, *Centaurea diffusa*, biological control, insects

#### **Introduction**

Spotted knapweed, *Centaurea maculosa* Lamarck, and diffuse knapweed, *Centaurea diffusa* Lamarck, are Eurasian plants that have become weed problems on western rangelands, particularly in the Pacific Northwest. Spotted knapweed is a perennial plant while diffuse knapweed is a biennial or short-lived perennial. Spotted knapweed, first reported in North America in 1893 (Groh 1944), now infests over 7 million acres, while diffuse knapweed, first reported in 1907 (Howell 1959), now infests over 3 million acres of rangeland and pasture in the western United States (Lacey 1989). Both weeds occur in many states but are particularly problematic in the western United States and Canada (Lacey 1989, Sheley et al. 1998).

The use of natural enemies to biologically control spotted and diffuse knapweed has been underway in North America since 1970 when the Eurasian flower head fly, *Urophora affinis*, was introduced into British Columbia (Harris 1980). Biocontrol efforts were initiated in the United States in 1973 when the same fly was introduced into Montana and Oregon (Story and Anderson 1978, Maddox 1982). To date, a total of 13 Eurasian natural enemies (all insects), extensively tested to prove that they will not attack non-target plants, have been introduced into North America for biological control of one or both of the two knapweed species. Eight of the insects attack knapweed flower heads while five attack the roots. This paper describes the 13 insects, their status, and overall progress of the biocontrol effort against spotted and diffuse knapweed in the western region of the United States.

#### **Biocontrol agents of spotted and diffuse knapweed**

1. *Urophora affinis* Frauenfeld - flower head fly. Larvae feed in the flower heads where they cause the formation of hard, woody galls in the receptacle tissue. The galls divert plant nutrients, resulting in reduced seed production in both attacked and unattacked flower heads on a plant. *Urophora affinis* generally has one generation per year although a small

percentage (approximately 7%) emerge in August and complete a second generation (Zwölfer 1970; Gillespie 1983; Story et al. 1992). The fly attacks the flower heads of both spotted and diffuse knapweed.

2. *Urophora quadrifasciata* (Meigen) - flower head fly. The biology of *U. quadrifasciata* is similar to that of *U. affinis* except that *U. quadrifasciata* forms papery galls in the ovary, attacks larger flower heads than does *U. affinis*, and generally has two generations per year (Harris 1980; Gillespie 1983). *Urophora quadrifasciata* was introduced into British Columbia in 1972 (Harris 1980), but not into the United States. However, by the early 1980s, the fly had dispersed into the United States and is now widely dispersed. The fly attacks the flower heads of both spotted and diffuse knapweed.

3. *Chaetorellia acrolophi* White and Marquardt - flower head fly. Larvae attack young seeds, florets, and parts of the seed head receptacle. The fly does not form galls and has two generations per year. The fly attacks the flower heads of both spotted and diffuse knapweed.

4. *Terellia virens* (Loew) - flower head fly. Larvae attack young seeds and occasionally feed on the seed head receptacle. The fly does not form galls. *Terellia virens* often has two generations, depending upon fall weather (Groppe and Marquardt 1989). The fly only attacks the flower heads of spotted knapweed.

5. *Metzneria paucipunctella* Zeller - flower head moth. Larvae feed on seeds and mine the receptacle. In addition, larvae bind seeds together with silk webbing which prevents dispersal of those seeds at maturity. The larvae will also attack and destroy other seed head insects, including larvae of the two flower head flies, *Urophora* spp. Destruction of up to 63 percent of *U. affinis* and *U. quadrifasciata* larva in attacked flower heads has been documented (Story et al. 1991). The moth is particularly vulnerable to extreme winter temperatures (Good et al. 1997) and, thus, is most successful in Oregon, Washington, and other areas with mild winters. The moth has one generation per year. Spotted knapweed is the moth's preferred host, but small numbers will also attack diffuse knapweed.

6. *Larinus minutus* Gyllenhal - flower head weevil. Larvae feed on knapweed seeds while adult weevils feed on knapweed leaves and stems. The weevil has one generation per year. Diffuse knapweed is the weevil's preferred host, but the weevil will also attack spotted knapweed.

7. *Larinus obtusus* Gyllenhal - flower head weevil. Larvae feed on knapweed seeds while adult weevils feed on knapweed leaves. The weevil has one generation per year. Spotted knapweed is the weevil's preferred host, but the weevil will also attack diffuse knapweed.

8. *Bangasternus fausti* Reitter - flower head weevil. Larvae feed on flower head florets and ovules. The weevil has one generation per year. The weevil attacks flower heads of both knapweed species.

9. *Agapeta zoegana* L. - root moth. Young larvae mine in the epidermal tissues of the root crown while older larvae mine in the cortex and endodermis tissues. The moth has one generation per year. Mass-rearing efforts have been conducted in Montana for 10 years to

hasten the distribution of the moth. Spotted knapweed appears to be the preferred host, but the moth is also established on diffuse knapweed in areas containing both plant species.

10. *Cyphocleonus achates* (Fahraeus) - root weevil. Larvae of this large weevil mine the root cortex while adults feed on knapweed leaves. Feeding by older larvae produces a gall-like enlargement of the root. The weevil has one generation per year. Dispersal is slow, because adults don't fly. Mass-rearing efforts have been conducted in Montana for 8 years to hasten the weevil's distribution. Spotted knapweed appears to be the preferred host, but the weevil is also established on diffuse knapweed in areas containing both plant species.

11. *Pelochrista medullana* (Staudinger) - root moth. The moth's biology is very similar to that of *A. zoegana*. Young larvae mine in the epidermal tissues of the root crown, while older larvae mine in the cortex and endodermis tissues. The moth has one generation per year. The moth attacks only the roots of spotted knapweed.

12. *Pterolonche inspersa* Staudinger - root moth. Larvae mine both in the epidermal tissues of the root crown and in the root core. The moth has one generation per year. The moth will attack the roots of both diffuse and spotted knapweed, but apparently prefers diffuse knapweed.

13. *Sphenoptera jugoslavica* Obenberger - root beetle. Young larvae feed in the leaf axil while older larvae burrow into the upper root. Feeding by the older larvae induces the formation of spindle-shaped root galls. Feeding by the larvae stops rosette growth and severely reduces the vigor of bolted plants. Adults feed regularly on knapweed leaves; heavy defoliation can kill seedlings and small first-year plants. The beetle has one generation per year. Diffuse knapweed appears to be the preferred host, but the beetle is also established on spotted knapweed.

The flower head moth and flies overwinter in the seed head, the flower head weevils overwinter in the soil, and the root insects overwinter in the root.

### **Status of biocontrol agents on spotted knapweed**

The status of biocontrol agents released against spotted knapweed is shown in Table 1. To date, the most successful biocontrol agents on spotted knapweed include *Urophora affinis*, *U. quadrifasciata*, *Agapeta zoegana*, and *Cyphocleonus achates*. Harris (1991) reported that *U. affinis* had reduced spotted knapweed seed production by 92 percent at locations in British Columbia. The two *Urophora* species have jointly reduced seed production by a minimum of 50 percent in Montana (Story et al. 1989). Both fly species are well established throughout the spotted knapweed-infested areas of the Pacific Northwest. *Urophora affinis* is the dominant species in most areas where the two flies coexist.

Regionally, *A. zoegana* and *C. achates* are spreading at a modest rate but are causing noticeable reductions in spotted knapweed density and vigor at several locations in western Montana. Knapweed plants at a site in western Montana with a high *A. zoegana* population had less above-ground biomass (43%), fewer stems per plant (29%), fewer capitula per plant (43%), and were shorter (18%) than knapweed plants in a control site (Story et al. 2000). *Agapeta zoegana* appears to do best in relatively dry sites.

Although no impact data have been obtained yet, *C. achates* may ultimately be the most effective agent against spotted knapweed. The weevil has caused noticeable changes in

knapweed density at several sites in western Montana. The biggest limitation to *C. achates* is its apparent inability to fly. The insect appears to do best in dry sites with large, scattered knapweed plants.

*Metzneria paucipunctella* is widely established in Idaho, Oregon and Washington but has been only minimally effective against spotted knapweed. The moth has failed to develop large populations due, apparently, to parasitoids and predaceous mites and limited cold tolerance. Each larva destroys about eight seeds per flower head in spotted knapweed (Story et al. 1991).

The two *Larinus* beetles appear promising but their population buildup has been slow. Increasing populations of *L. obtusus* in western Montana may enable the initiation of redistribution efforts in 2001.

*Chaetorellia acrolophi* and *Terellia virens* are established in several states. In Oregon, the two flies are most successful in areas where *Larinus* spp. are not present. In Montana, the two flies appear to be severely hindered by high *U. affinis* densities.

*Sphenoptera jugoslavica* is well established on spotted knapweed in Oregon but has had no impact on the plant.

*Bangasternus fausti*, *Pelochrista medullana*, and *Pterolonche inspersa* have not fared well, to date, in the United States on spotted knapweed. The only known establishment of *B. fausti* on spotted knapweed is in Oregon where it is established in limited numbers, while the only established colony of *P. medullana* in the United States exists in western Montana. *Pterolonche inspersa* has failed to establish on spotted knapweed at all release sites in the United States.

### **Status of biocontrol agents on diffuse knapweed**

The status of biocontrol agents released against diffuse knapweed is shown in Table 1. The most successful biocontrol agents on diffuse knapweed to date have been *Urophora affinis*, *U. quadrifasciata*, *Sphenoptera jugoslavica*, and *Larinus minutus*. The combined attack by the two *Urophora* species and *S. jugoslavica* has resulted in a 98 percent reduction in seed numbers at one site in British Columbia (Harris and Shorthouse 1996). The two *Urophora* species are well established throughout the diffuse knapweed-infested areas of the Pacific Northwest.

*Sphenoptera jugoslavica* is well established on diffuse knapweed throughout the Pacific Northwest. The beetle causes noticeable stunting of diffuse knapweed plants in some areas but has had no measurable impact on plant density in the United States.

*Larinus minutus* is the most impressive agent, to date, against diffuse knapweed. The insect is having a significant impact on the plant growth and density at many locations in the Pacific Northwest. In addition to seed destruction by the larvae, adults do extensive feeding on growing plants in the spring, which typically results in complete destruction of all growing knapweed plants in the vicinity of the original insect release. Especially impressive is the fact that the adults also destroy seedlings, resulting in minimal recruitment of new plants. Diffuse knapweed plants under attack by *L. minutus* typically turn a characteristic blue-green color, have very few leaves, and often have distorted growth. Heavily-attacked plants can also be brownish in color due to adult feeding and excessive excrement. The insect develops large populations within 3 to 5 years and disperses rapidly to new areas.

*Agapeta zoegana* and *Cyphocleonus achates* are both established on diffuse knapweed in very limited numbers. Attack on diffuse knapweed by both insect species is generally limited to large plants growing adjacent to spotted knapweed.

*Metzneria paucipunctella* will attack flower heads of diffuse knapweed in very small numbers (< 5%), but spotted knapweed is obviously the preferred host plant.

*Bangasternus fausti* is established in limited numbers on diffuse knapweed in California and Oregon.

*Pterolonche inspersa* has been recovered on diffuse knapweed in very small numbers at one site in Oregon.

*Larinus obtusus*, *Chaetorellia acrolophi*, *Terellia virens*, and *Pelochrista medullana*, released only on spotted knapweed, have not established on diffuse knapweed in the United States.

## Conclusion

Harris and Cranston (1979) suggested that the establishment of six natural enemy species would be needed for biological control to be effective against the two knapweed species in North America. Using that suggestion as a general guideline, a primary objective of the knapweed biocontrol effort at the time of the last knapweed symposium (1989) was to introduce all of the natural enemy species that appeared promising as biocontrol agents. That objective was achieved in 1992 when the thirteenth and final natural enemy species targeted for biocontrol of the two knapweeds was introduced into the United States.

Many of the introduced biocontrol agents are increasing quite slowly so the firm establishment of six natural enemies on the two knapweeds has not yet been achieved. Nevertheless, biocontrol prospects against each weed appear promising. *Larinus minutus* appears to be an exceptional agent and will likely have a significant impact on diffuse knapweed in the Pacific Northwest. Similarly, *Agapeta zoegana* and *Cyphocleonus achates* are showing great potential against spotted knapweed. Other agents may prove to be effective once their numbers increase, particularly at sites where agents like *L. minutus*, *A. zoegana*, or *C. achates* are already well established.

There is no question that biological control is going to play an important role in the ultimate management of the knapweeds. However, biocontrol does have limitations and, therefore, will not be a "cure-all." Successful management of spotted and diffuse knapweed will be a long-term effort involving the combined use of all available control methods and improved land management practices in an integrated approach.

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Table 1. Status of biocontrol agents in the western United States against spotted and/or diffuse knapweed

| Biocontrol agent                | Date released | Status of biocontrol agent in 9 western states <sup>1,2</sup> |   |
|---------------------------------|---------------|---|---|
|                                 |               | Spotted knapweed  | Diffuse knapweed                                  |
| <i>Urophora affinis</i>         | 1973          | W --- CA, CO, ID, MT, OR,<br>WA, WY<br>L --- NV, UT           | W --- CA, CO, ID, MT, OR,<br>WA, WY<br>L --- UT   |
| <i>Urophora quadrifasciata</i>  | 1980          | W --- CA, CO, ID, MT, OR,<br>UT, WA, WY<br>L --- NV           | W --- CO, ID, MT, OR, UT,<br>WA, WY<br>L --- CA   |
| <i>Metzneria paucipunctella</i> | 1980          | W --- ID, OR, WA<br>L --- CO, MT                              | L --- MT, OR, WA                                  |
| <i>Sphenoptera jugoslavica</i>  | 1980          | L --- MT, OR<br>U --- CO, NV, UT, WY                          | W --- CA, ID, OR, WA,<br>L --- CO, MT, NV, UT, WY |
| <i>Agapeta zoegana</i>          | 1984          | L --- CA, CO, ID, MT, OR,<br>UT, WA, WY                       | L --- CO, MT, UT, WY<br>U --- OR                  |
| <i>Pelochrista medullana</i>    | 1984          | L --- MT<br>U --- OR  |   |
| <i>Cyphocleonus achates</i>     | 1988          | W --- CO<br>L --- CA, MT, NM, OR,<br>UT, WA, WY               | L --- CO, MT, OR, UT,<br>WA, WY                   |
| <i>Pterolonche inspersa</i>     | 1988          | U --- MT, OR  | L --- OR<br>U --- ID                              |
| <i>Bangasternus fausti</i>      | 1990          | L --- OR<br>U --- ID, MT                                      | L --- CA, OR<br>U --- ID, MT, UT                  |
| <i>Larinus minutus</i>          | 1991          | W --- CA, WY<br>L --- CO, ID, MT, NV, OR,<br>UT, WA           | W --- WY<br>L --- CA, CO, MT, NV, OR,<br>UT, WA   |
| <i>Larinus obtusus</i>          | 1992          | L --- MT, OR, WA, WY<br>U --- CO                              |   |
| <i>Chaetorellia acrolophi</i>   | 1992          | L --- CO, OR, WA, WY<br>U --- MT                              |   |
| <i>Terellia virens</i>          | 1992          | L --- CA, OR<br>U --- MT, WA, WY                              |   |

<sup>1</sup> Data from E. Coombs, Western USA Biological Control of Weeds Database.

<sup>2</sup> W = Widespread, L = Established at limited sites, U = Unknown status, CA = California, CO = Colorado, ID = Idaho, MT = Montana, NV = Nevada, OR = Oregon, UT = Utah, WA = Washington, WY = Wyoming.

## Considerations for Resuming Foreign Exploration for Natural Enemies of Spotted and Diffuse Knapweed

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### Abstract

Several introduced biological control agents occur in high numbers at sites in Washington, Oregon, and Montana where diffuse knapweed populations appear to be decreasing. However, similar agents appear to have had little success at many spotted knapweed sites. Do we simply need to wait longer for these to take effect, or do we need to find additional agents? The introduced agents came from “*Centaurea maculosa*” in central Europe (Switzerland, Austria, Hungary, Romania, Greece). However, *C. maculosa* in Europe is a monocarpic biennial diploid (it grows two seasons, flowers once and dies;  $2n = 18$ ). The North American plant is a polycarpic, perennial tetraploid (it grows and flowers many years;  $2n = 36$ ). A recent taxonomic revision of a small part of the genus *Centaurea* by Jörg Ochsman indicates that the perennial tetraploid plant (which he names *C. stoebe* L. subsp. *micranthos*) originates in eastern Europe (southern Russia to Hungary and Bulgaria). Thus, it is likely that the center of diversity of agents attacking this plant occurs in eastern Europe, which had not been explored because of the Cold War. Climatic analysis indicates that areas in western Montana, where spotted knapweed infestations are greatest, are similar to Ukraine, southern Russia and central Turkey. Thus it appears that we are currently using insects that come from a milder climate and a different, less vigorous plant. There is also an important ecological niche that has not yet been filled – none of the currently established agents attack the rosette foliage or root crown. These plant structures are vulnerable to attack for at least one year before the plant can reproduce. Some potential candidates that attack these structures were reported in previous explorations by H. Müller. Unlike the established root-feeding insects, which are insulated by several centimeters of soil, insects located near the soil surface can benefit from solar heating, an important environmental factor in the semiarid western USA, which can speed up development rate in the spring and fall, and which should increase insect population growth and impact.

Key words: Biological control, foreign exploration, climate matching, taxonomy, *Centaurea maculosa*, *Centaurea diffusa*

### Introduction

Spotted and diffuse knapweeds are closely related alien plants from Europe that are highly invasive in semiarid regions of the northwestern continental United States and southwestern Canada (e.g., Harris and Cranston 1979; Maddox 1979). They have been the target of control programs for over 40 years, including the use of herbicides, livestock grazing, cultural control, grass competition and biological control (e.g., Sheley et al. 1998 and references therein). Biological control researchers have introduced 13 agents that had been identified during exploration of Europe in the 1960s through the 1980s (e.g., Müller-Schärer and Schroeder 1993, Story and Piper 2001). Do we have enough agents now to reduce these weeds to innocuous population levels? If these agents are not sufficient, are there any prospects for discovering more agents? The purpose of this paper is to review the current state of biological control, in complement to that of Story and Piper (2001), and



discuss prospects for finding additional agents to complement those already established in North America.

### **Established agents**

Thirteen species of insect have been released as biological control agents of weeds in North America (Rees et al. 1996), and at least seven are well established and spreading (see Story and Piper 2001):

- Agapeta zoegana* L. - sulphur knapweed moth (root exterior),
- Cyphocleonus achates* (Fahraeus) - knapweed root weevil (root interior),
- Larinus minutus* Gyllenhal - lesser knapweed flower weevil,
- Metzneria paucipunctella* Zeller - knapweed seedhead moth,
- Sphenoptera jugoslavica* Obenberger - bronze knapweed root-borer (root galling beetle),
- Urophora affinis* Frauenfeld - banded dall fly,
- U. quadrifasciata* (Meigen) - UV knapweed seed head fly.

All of these agents readily attack both spotted and diffuse knapweed. Of these agents, the seed head flies and seed head weevils are easily collected for redistribution (Story 1984; Kashefi and Sobhian 1998). *Sphenoptera jugoslavica* can be collected in abundance, under specific favorable conditions (Lang et al. 1998). But, *A. zoegana* and *C. achates* are generally more difficult to collect in the field (Story et al. 1999), so they are also multiplied in caged garden insectaries (Story et al. 1994; Story et al. 1996). The moth is too fragile for sweep-netting, and light traps tend to collect mainly males (Story et al. 2001), although the latter method has been used successfully in Oregon (E. Coombs, personal communication). *Cyphocleonus achates* has generally been difficult to collect in the field (Story et al. 1999). In Oregon, adults are easily collected from under the leaves of rosettes in late summer and early fall (E. Coombs, personal communication). Availability has limited the rate at which these two insects have been distributed.

A petition for a permit to introduce the leaf gall mite, *Aceria centaureae* (Nalepa), recently has been submitted to the USDA-APHIS Technical Advisory Group For Biological Control Agents of Weeds (TAG) (J. Littlefield, personal communication). This agent appears to be specific to the subtribe Centaureine, but must undergo more host plant specificity testing before it is likely to be approved.

### **Impact**

Diffuse knapweed populations recently appear to be declining at many sites in Washington, Oregon and Montana (personal observation; G. L. Piper, E. M. Coombs and R. F. Lang, personal communication). This decline has been attributed primarily to the impact caused by high densities of the two *Urophora* flies, *L. minutus* and *S. jugoslavica*. The seed head flies greatly reduce seed production, but generally not enough to provide adequate control (Cloutier and Watson 1989, Myers et al. 1989). Although *S. jugoslavica* is widespread in Washington and Oregon, it also has not been considered to provide sufficient control of the weed (Powell 1989). The impact of *L. minutus* adults feeding on rosettes in the spring is suspected to be a deciding factor in the decline of diffuse knapweed (G. L. Piper and R. F. Lang, personal communication), although this has not been proven. *Cyphocleonus achates* also appears to have substantial impact, directly killing bolting diffuse knapweed at a site in Montana (unpublished data), despite the belief that this insect is generally associated more with spotted than diffuse knapweed. Unfortunately, there is little published documentation of these declines in weed density or hard data showing the direct impact of these agents on

diffuse knapweed [except for *S. jugoslavica* impact (Powell and Myers 1988) and *Urophora* impact (Powell et al. 1989)].

Spotted knapweed appears to have responded much less to the impact of established biological control agents. The two *Urophora* seed head flies are widely established, but they have not reduced knapweed populations by themselves (Müller and Schroeder 1989, 1993). Story et al. (2000) documented apparent reductions in the density and size of plants in the presence of an increasing population of the root-feeding moth, *A. zoegana*, on floor of the Bitterroot Valley, Montana (20-40% of the plants were infested). A separate survey of 13 sites in western Montana where *A. zoegana* and/or *C. achates* had been released 4-6 years previously failed to detect significant impacts, although this was not the principal objective of the study (Clark 2000). Most of these sites were also at higher elevation, on sloped terrain and adjacent to ponderosa pine forests, all of which could have reduced growing degree days available for the root-feeding insects. Fewer degree days means later emergence, shortened time favorable to oviposition, and slower population growth.

*Agapeta zoegana* and *C. achates* populations are clearly expanding in the Bitterroot Valley, Montana (J. M. Story unpublished data), but spotted knapweed populations have not decreased as dramatically as those of diffuse knapweed observed elsewhere. This could be because spotted knapweed is more vigorous (perennial) than diffuse knapweed (biennial), because of a larger persistent seedbank, or because of colder climate which reduces the growth rate of insect populations. In an interesting challenge to current dogma, a one-year garden plot study and greenhouse experiment supported the hypothesis that *A. zoegana* actually increases the fitness of spotted knapweed in the presence of grasses and mycorrhizae (Callaway et al. 1999, Marler et al. 1999). A garden plot experiment to measure the interactions of a herbicide and *C. achates* in Montana failed to detect an impact of the root-borer on knapweed (Jacobs et al. 2000). At the very least, these studies confirmed that *A. zoegana* and *C. achates* do not cause dramatic immediate negative effects on spotted knapweed, which agrees with results of earlier greenhouse experiments (Müller 1989a, Müller-Schärer 1991, Steinger and Müller 1992).

Some populations of *L. minutus* attack spotted knapweed (see Story and Piper 2001), and the *L. obtusus* populations in the Bitterroot Valley, Montana and Hood River, Oregon have recently become large enough to be collectable. If either of these species damages spotted knapweed rosettes in the spring, as has been observed on diffuse knapweed, then these species could play an important role; however, such damage needs to be documented.

### **Plant taxonomy**

The genus *Centaurea* comprises about 600 species and is taxonomically difficult. Although the North American literature generally refers to spotted knapweed as "*Centaurea maculosa*", it is a polycarpic perennial that best corresponds to the European plant *Centaurea stoebe* L. subsp. *micranthos* (Ochsmann 2000), which was synonymized with *Centaurea biebersteini* DC. This plant is a tetraploid (twice the usual number of chromosomes,  $2n = 36$ ) which clearly distinguishes it from the diploid monocarpic biennial, *Centaurea maculosa* Lamarck (which was synonymized to *Centaurea stoebe* L. subsp. *stoebe* by Ochsmann;  $2n = 18$ ). Whether all the spotted knapweed plants in North America are tetraploid and belong to the same subspecies remains to be fully documented. Ongoing genetic studies using molecular markers will help to resolve this question (Hufbauer et al. 2001). Nevertheless, according to Ochsmann (2000), *C. s. micranthos* originated in southeastern Europe (southern Russia to Hungary, Bulgaria and Turkey) and has recently invaded central Europe (Figure 1). Furthermore, Groh (1940) argued that the species was introduced to North America by seed

from Turkestan. The center of diversification of the genus is also considered to be southern USSR (Dostál 1976). Nevertheless, most of the insect biological control agents introduced to North America came from western and southern Europe, evidently collected from plants such as *C. s. stoebe*, *C. vallesiaca* Jordan and *C. s. serbica* (Prodan) Ochsmann rather than from *C. s. micranthos* (Müller and Schroeder 1989). Because many of the introduced biological control agents attack both spotted and diffuse knapweed, the mistaken identity of the North American target plant may not be crucial regarding host plant acceptance. However, the differences in the life histories of the plants can affect the impact of biological control agents (Müller 1989a). Although foreign exploration was previously limited to western and central Europe for political reasons, eastern Europe and western Asia are now accessible. A renewal of the foreign exploration for agents of spotted and diffuse knapweeds is planned, and Ukraine, Russia and Turkey will be surveyed beginning this year (Sforza et al. 2001).

### **Climatic similarity**

It has been well documented that some of the insects that overwinter inside the seed heads of spotted and diffuse knapweed are vulnerable to low winter temperatures (Story et al. 1993, Good et al. 1997, Nowierski et al. 2000). Root-feeding insects, which begin developing as larvae in the fall and complete development the following spring and summer, are probably limited more by an insufficiency of accumulated degree days (which delays adult emergence) than by minimum winter temperatures. A geographic information system (GIS) phenology model based on observed emergence dates indicated that many locations in Montana may have too few accumulated degree-days to favor the establishment and increase of the root weevil *C. achates* (R. Hansen, unpublished data). Based on the assumption that sites in western Montana where spotted knapweed densities are highest may be too cold for most of the agents already introduced, a study was conducted to determine what region in Eurasia had the most similar climate (Rice et al. 2000; unpublished data). Knapweed sites in western Montana most closely matched regions in Ukraine and central Turkey, which is also where *C. s. micranthos* occurs). Most of the introduced insect agents came from central and southern Europe, which has a milder climate than western Montana.

### **Ecological niches**

It has been suggested by several scientists who have worked on biological control of spotted and diffuse knapweeds that a combination of agents will be needed to help bring these plants under control in North America (Cloutier and Watson 1989, Harris 1989, Myers et al. 1989, Powell 1989, Müller-Schärer and Schroeder 1993). It appears that the two *Urophora* flies and at least one of the *Larinus* species substantially reduce seed production (Harris and Cranston 1979, Harris 1980, Roze 1981, Groppe et al. 1990). Müller (1989c), who did much of the European exploration for agents, identified five different parts of the root that could be attacked by insects, and Müller et al. (1989) recommended finding agents that attack each. The established agents, *A. zoegana*, *C. achates* and *S. jugoslavica*, occupy only two of these root niches. Furthermore, it has been suggested, based on simulation models and ecological analysis, that agents attacking the rosette stage should be more effective in reducing knapweed density than seed-feeding agents (Müller 1989b, Powell 1989, Jacobs and Sheley 1998). Some European insects attacking the rosette buds (*Stenodes straminea* Haw., *Pegohylemyia centaureae* Hennig) and root collar (4 *Apion* species, 1 *Cheilisia* sp.) have already been observed (Müller 1989c), but most need further investigation. In addition to attacking new plant niches, such insects may be more successful in cold habitats because they are exposed to a greater accumulation of degree days than

insects located further underground because of solar heating, which plays an important role in microclimate in the northwestern U.S.A.

### Pathogens

There has been some foreign exploration for plant pathogens of *C. stoebe sensu lato* (e.g., Watson and Clement 1986), and the rust pathogens *Puccinia centaureae* D.C. and *Puccinia jaceae* var. *diffusae* have been reported on diffuse knapweed in North America (Savile 1973, Mortenson et al. 1989). But, pathogens seem to have been largely unexplored, especially those species attacking immature plants.

### Conclusion

Although we may now have enough effective agents to reduce diffuse knapweed populations to acceptable levels, it does not yet appear to be the case for spotted knapweed. If the population of *L. obtusus* were to begin increasing as rapidly as that of *L. minutus*, this would provide renewed encouragement for controlling spotted knapweed with the currently available agents. Barring this, we lack agents that attack the foliage and the root crown of rosettes during the fall and spring, which appear to be critical periods for accumulating carbohydrate reserves, and which population dynamic models suggest is an important plant stage to attack. The most promising geographic region to explore, based on similar climate and presence of the target plant is in southern Russia and central Turkey. The first priority should be to find agents that attack the rosettes in the spring or fall, especially those that attack the leaves, foliar meristem and root collar.

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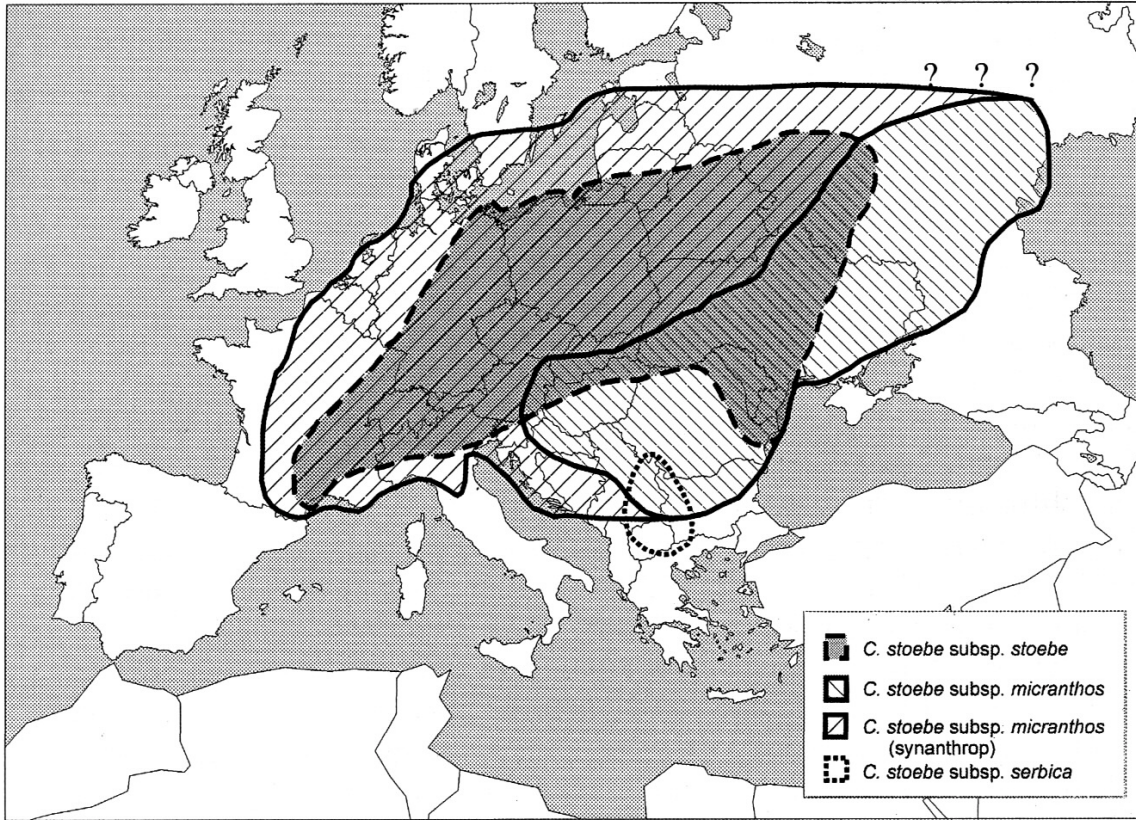
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(Möglicherweise muß das Gesamtareal nach Osten erweitert werden; s. Kap. 1.1.1.3)

Abb. 13: Verbreitung der Unterarten von *C. stoebe*

Figure 1. Distribution of *Centaurea stoebe stoebe* (= diploid *C. maculosa*) and *C. stoebe micranthos* (= tetraploid weed in North America). The area labelled "*C. stoebe micranthos* (synanthrop)" represents recent spread of this plant into western Europe. Copied from Ochsmann (2000), with permission.



## Prescription Grazing for *Centaurea* Control on Rangelands

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### Abstract

Spotted knapweed (*Centaurea maculosa*) is an aggressive short-lived perennial weed that typifies the impact of noxious weeds on wildland ecosystems. Several decades of research and field experiences have not yielded an effective and economically feasible control system for spotted knapweed. It has long been recognized that weed management systems on rangelands must incorporate grazing management plans to be effectively implemented. However, we propose a shift in emphasis from working weed control programs around grazing management plans to actively employing livestock in the battle against weeds. Grazing could be honed into a highly effective weed management tool with precise application based on an understanding of plant-herbivore interactions. Recent success in the use of sheep and goats to control some rangeland weeds, such as leafy spurge (*Euphorbia esula*), has fueled interest in grazing for weed control. Carefully managed grazing holds potential for weed control in situations where traditional methods (e.g., mechanical, cultural, biological, or chemical) are restricted by environmental or economic constraints. We are currently conducting research on the potential of strategic sheep grazing to control spotted knapweed. We will discuss: 1) the season and plant age when spotted knapweed is most susceptible to damage by grazing, 2) the forage value of spotted knapweed, and 3) the stocking rate necessary to accomplish knapweed control. We will present a framework for prescription grazing to control spotted knapweed, revealing recent findings, and synthesizing information on grazing effects on other *Centaurea* species.

Key words: Prescription Grazing, Grazing Management, Spotted Knapweed, *Centaurea*

It has long been recognized that weed management systems on rangelands must incorporate grazing management plans to be effectively implemented (Sheley et al. 1996). However, grazing could be more effectively employed in the battle against weeds if the specific time of grazing and necessary stocking rate were known for specific weeds. Recent success in the use of sheep and goats to control some rangeland weeds, such as leafy spurge (*Euphorbia esula*), has fueled interest in grazing for weed control (Walker et al. 1994, Olson 1999). Carefully managed grazing holds potential for weed control in situations where traditional methods (e.g., mechanical, cultural, biological, or chemical) are restricted by environmental or economic constraints (Olson and Lacey 1994). Furthermore, livestock grazing has one distinct advantage over other control methods; in the process of controlling a noxious plant, grazing animals convert the weed into a saleable product (Walker et al. 1994).

Livestock grazing, like any tool, can be misapplied and cause harm instead of repair. Overgrazing has often been implicated in the spread of noxious weeds. However, we suggest that grazing could be honed into a highly effective weed management tool with precise application based on an understanding of plant-herbivore interactions. Effective grazing programs for weed control require a clear statement of the kind of grazing animal, timing, and rate of grazing necessary to reduce noxious plants and maintain healthy rangeland

ecosystems (Mosley 1996). A successful grazing prescription should: 1) cause significant damage to the target plant (Walker et al. 1992); 2) limit irreparable damage to the surrounding vegetation (Walker et al. 1992, Olson and Lacey 1994); 3) be consistent with sheep production goals (Olson and Lacey 1994, Mosley 1996) ; and 4) be integrated with other control methods as part of an overall pest management strategy (Sheley et al. 1996).

Spotted knapweed (*Centaurea maculosa*) is a promising candidate for control by prescription grazing because defoliation can negatively affect knapweed growth (Olson et al. 1997) and knapweed is readily grazed by livestock (Olson and Lacey 1994, Sheley et al. 1998). If properly applied, grazing could shift the competitive edge from spotted knapweed to the native plant species. This requires an understanding of phenological stage when spotted knapweed is most sensitive to defoliation and when it is most palatable to grazing animals relative to native vegetation. In 1999, we initiated a study at the USDA-ARS U.S. Sheep Experiment Station near Dubois, Idaho, USA to examine: 1) knapweed response to defoliation; 2) potential forage value of knapweed; 3) the effect of phenological stage on grazing preference; and, 4) the relative preference of knapweed in a native sagebrush-grassland community.

### **Plant response to defoliation**

Developing effective grazing systems to control spotted knapweed requires that grazing be strategically applied to maximize the detrimental effect to knapweed and simultaneously minimize negative influences on associated vegetation. Evaluating individual plant responses to different clipping regimes will provide insight to achieve this balance. In May 1999, 96 circular plots (0.1 m<sup>2</sup>) were located in an area infested with spotted knapweed. Plots contained 1 to 3 knapweed plants and the associated vegetation was composed mainly of perennial grasses and forbs. The 96 plots were randomly assigned to 1 of 3 phenological stages (rosette, bolt, or flowering) and 1 of 3 defoliation treatments (clipping only spotted knapweed, clipping only the associated vegetation, or clipping both the spotted knapweed and associated vegetation) and an undefoliated control within each phenological stage. These treatment combinations were designed to approximate potential herbivory scenarios for grazing spotted knapweed-dominated communities. Each phenological and clipping treatment combination was replicated 8 times. In May 2000, three additional sites with 96 plots each were established but these plots were not included in the following analysis.

In each plot, the basal area and number of rosettes of spotted knapweed and the number and basal area of the perennial and annual forbs and grasses in the associated vegetation were measured before and after each growing season. The number of flower buds on each knapweed plant was counted at the end of the growing season. Seedheads were subsampled on each plant and seed number and percent germination were determined in a germination chamber at the Northern Great Plains Research Lab in Mandan, North Dakota, USA.

Basal area of spotted knapweed was significantly affected by clipping treatment; however, defoliation did not affect the density of spotted knapweed or the surrounding vegetation. The basal area of spotted knapweed increased less when spotted knapweed was defoliated compared to non-defoliated controls. Defoliating the spotted knapweed plants, either alone or in combination with associated vegetation, significantly reduced seedhead production (Table 1). There were fewer seedheads produced when defoliation occurred in the flowering phenological stage than in the bolting or rosette stages. Clipping spotted knapweed plants significantly reduced germination percent in the flowering phenological stage but not in the bolting or rosette stages.

Table 1. The number of seedheads produced per spotted knapweed plant under the clipping treatments. Treatments with different letters are significantly different at the  $P < 0.05$ .

| Clipping Treatment                        | Seedheads per Plant |
|---|---------------------|
| Spotted Knapweed & Surrounding Vegetation | 1.04 <sup>a</sup>   |
| Spotted Knapweed Only                     | 0.86 <sup>a</sup>   |
| Surrounding Vegetation Only               | 18.62 <sup>b</sup>  |
| Control (no clipping)                     | 18.91 <sup>b</sup>  |

### **Potential forage value of knapweed**

Converting grazing from a ubiquitous rangeland practice to a powerful tool for weed control will require information on potential grazing value of the target plant, and the effects of prescription grazing on livestock production. In 1999 and 2000 we collected knapweed samples from 5 sites in a knapweed-dominated sagebrush steppe community near the U.S. Sheep Experiment Station. We also separated plants into leaf, stem, and flowering portions in 2000. Samples were dried and prepared for laboratory chemical analysis to assess nutritive value (crude protein, neutral detergent fiber, acid detergent lignin, 48-hr digestibility, and total non-structural carbohydrates).

Spotted knapweed has moderate forage quality with low fiber and high digestible dry matter early in the season (Table 2). Digestibility, crude protein and non-structural carbohydrates decreased as plants matured. Knapweed chemical composition was not affected by size or age of plant

Decreasing forage value as season progresses was a result of decreasing leaf:stem ratios and the appearance of flowers late in the season. Leaves on mature plants and new rosettes do not vary in quality throughout the growing season (Table 3). However, stems become more fibrous and less digestible as the season progressed. Lignin content also increased as plants matured, but it was equally present in leaves, stems, and flowers. Flowers appear in late summer and possess forage quality intermediate to leaves and stems.

### **Grazing preference for knapweed**

To develop an effective grazing prescription for weed control it is necessary to know the season or phenological stage when weed plant is most palatable and most likely to be consumed. To establish the season in which knapweed is most palatable, we conducted a cafeteria trial with sheep offered spotted knapweed in the rosette, bolting, and flowering stages. Foliage was collected in 1999, dried at 55°C, and stored in a cool dry room. In the summer of 2000, 12 ewes familiar with knapweed were individually penned and simultaneously offered 75 g. of each knapweed growth stage. Four preference trials were conducted for each sheep.

In the first trial, ewes expressed greatest preference for rosette foliage followed by bolting and flowering knapweed. However, in future trials sheep expressed variable preference between rosette and bolting foliage, though flowering was consistently least preferred. Sheep readily consumed knapweed in all growth stages suggesting that knapweed is an acceptable forage.

Table 2. Forage quality parameters (mean  $\pm$  SEM) of spotted knapweed collected from sagebrush steppe rangelands in southeastern Idaho in 1999. Three age-size classes were examined. Immature, plants in their first season of growth, Medium Mature, plants with fewer than 5 stems from previous year's growth, and Large Mature, plants with 5 or more stems from previous year's growth.

| Month | Age-Size Class | % dry matter  |                         |                |                        |                              |
|-------|----------------|---------------|-------------------------|----------------|------------------------|------------------------------|
|       |                | Crude Protein | Neutral Detergent Fiber | Lignin         | In vitro Digestibility | Non-structural Carbohydrates |
| May   | Immature       | --            | 29.4 $\pm$ 0.6          | 7.5 $\pm$ 0.4  | 72.7 $\pm$ 1.7         | 16.2 $\pm$ 0.3               |
|       | Medium Mature  | 16.5          | 35.0 $\pm$ 0.8          | 13.2 $\pm$ 0.7 | 70.5 $\pm$ 1.3         | 17.6 $\pm$ 0.1               |
|       | Large Mature   | 18.3          | 33.9 $\pm$ 1.0          | 11.6 $\pm$ 1.1 | 69.3 $\pm$ 1.1         | 16.3 $\pm$ 0.2               |
| June  | Immature       | 14.5          | 34.8 $\pm$ 1.3          | 12.3 $\pm$ 1.4 | 72.1 $\pm$ 2.9         | 23.2 $\pm$ 0.5               |
|       | Medium Mature  | 10.7          | 37.8 $\pm$ 0.9          | 13.9 $\pm$ 1.3 | 67.5 $\pm$ 1.4         | 22.2 $\pm$ 0.8               |
|       | Large Mature   | 10.2          | 37.7 $\pm$ 0.5          | 12.0 $\pm$ 0.6 | 67.9 $\pm$ 1.7         | 26.3 $\pm$ 0.9               |
| July  | Immature       | 6.8           | 41.7 $\pm$ 1.2          | 7.6 $\pm$ 0.2  | 59.4 $\pm$ 1.5         | 13.7 $\pm$ 0.2               |
|       | Medium Mature  | 6.8           | 41.7 $\pm$ 0.9          | 7.5 $\pm$ 0.8  | 63.2 $\pm$ 0.8         | 12.5 $\pm$ 0.2               |
|       | Large Mature   | 7.5           | 45.8 $\pm$ 1.1          | 7.0 $\pm$ 0.2  | 58.1 $\pm$ 1.4         | 11.5 $\pm$ 0.2               |
| Sept. | Immature       | 5.1           | 53.3 $\pm$ 0.4          | 9.6 $\pm$ 0.5  | 48.4 $\pm$ 1.5         | 10.9 $\pm$ 0.2               |
|       | Medium Mature  | 3.8           | 58.2 $\pm$ 1.6          | 9.9 $\pm$ 0.7  | 42.6 $\pm$ 1.4         | 10.0 $\pm$ 0.1               |
|       | Large Mature   | 3.4           | 62.7 $\pm$ 1.0          | 9.8 $\pm$ 0.3  | 40.3 $\pm$ 1.2         | 9.5 $\pm$ 0.2                |
|       | Fall Rosette   | --            | 28.1 $\pm$ 0.6          | 7.1 $\pm$ 0.5  | 64.0 $\pm$ 1.0         | 18.2 $\pm$ 0.3               |

### Grazing effects and utilization patterns of knapweed

Carefully controlled studies on individual plants and animals are necessary to identify opportunities for prescription grazing. However, the viability of grazing for weed control must be tested with grazing animals and noxious plants in natural settings. We established a grazing trial in summer 2000 to examine stocking rate and season when knapweed is most susceptible to damage by grazing. Grazing trials were conducted on private rangeland in eastern Idaho near the U.S. Sheep Experiment Station. Two rangeland sites (22 ha each) were fenced and divided into 21 paddocks. Dry ewes were grazed at 2 stocking rates for 4 days in the rosette, bolting, or flowering growth stages with appropriate ungrazed controls. In each paddock, 24 sampling plots (1830 cm<sup>2</sup>) were randomly placed and permanently marked along 3 transects. Spotted knapweed density and canopy cover of spotted knapweed, other forbs, grasses, shrubs and bare ground were assessed at the beginning and end of the growing season to track community changes. The number of knapweed flowers produced was also recorded at the end of the season to indicate effects of grazing on reproductive

potential. We measured biomass before, during, and after each 4-day grazing trial to determine utilization experienced by knapweed, other forbs, and grasses.

Table 3. Forage quality parameters (mean  $\pm$  SEM) of rosette and mature spotted knapweed plants collected from sagebrush steppe rangelands in southeastern Idaho in 2000. Foliage from mature plants was separated into portions of leaves, stems, and flowers.

| Month  | Plant Part | % dry matter  |                         |                |                        |
|--------|------------|---------------|-------------------------|----------------|------------------------|
|        |            | Crude Protein | Neutral Detergent Fiber | Lignin         | In vitro Digestibility |
| May    | Rosette    | --            | 29.7 $\pm$ 1.4          | 6.6 $\pm$ 0.5  | 73.5 $\pm$ 1.9         |
|        | Leaf       | --            | 20.9 $\pm$ 0.4          | 4.0 $\pm$ 0.3  | 72.1 $\pm$ 0.8         |
| June   | Rosette    | 11.0          | 24.5 $\pm$ 0.4          | 5.4 $\pm$ 0.3  | 71.0 $\pm$ 1.0         |
|        | Leaf       | 11.1          | 32.3 $\pm$ 1.6          | 6.9 $\pm$ 0.3  | 70.7 $\pm$ 1.6         |
|        | Stem       | 4.5           | 50.2 $\pm$ 0.9          | 5.6 $\pm$ 0.1  | 56.8 $\pm$ 1.1         |
| July   | Rosette    | 8.5           | 29.3 $\pm$ 0.4          | 7.7 $\pm$ 0.8  | 64.4 $\pm$ 1.2         |
|        | Leaf       | 8.4           | 30.6 $\pm$ 0.6          | 7.8 $\pm$ 0.4  | 67.7 $\pm$ 1.6         |
|        | Stem       | 1.9           | 57.3 $\pm$ 1.3          | 8.0 $\pm$ 0.3  | 41.9 $\pm$ 1.3         |
|        | Flower     | 8.1           | 43.1 $\pm$ 0.6          | 8.6 $\pm$ 0.6  | 54.0 $\pm$ 1.3         |
| August | Rosette    | 8.8           | 31.0 $\pm$ 1.5          | 8.7 $\pm$ 0.5  | 64.7 $\pm$ 0.6         |
|        | Leaf       | 5.6           | 31.6 $\pm$ 0.3          | 10.2 $\pm$ 0.9 | 62.3 $\pm$ 1.3         |
|        | Stem       | 1.6           | 66.2 $\pm$ 0.9          | 9.5 $\pm$ 0.3  | 35.8 $\pm$ 0.9         |
|        | Flower     | 7.7           | 54.4 $\pm$ 0.6          | 9.7 $\pm$ 0.7  | 44.2 $\pm$ 0.5         |

Few changes in community structure or composition were noted in this first season of research. No consistent differences were observed between grazed and ungrazed paddocks in canopy cover of knapweed, other forbs, grasses, or shrubs, except there was a tendency for grazing to reduce cover of native forbs on one site. The number of flowers per plot was slightly lower in grazed paddocks particularly when grazing occurred during the rosette stage. The density of young knapweed plants was slightly lower in paddocks grazed during the flowering stage than controls. Therefore, early season grazing may affect flower production, while grazing later in the year may reduce density of young plants.

Knapweed experienced greater than 40% utilization regardless of grazing season or stocking rate (Table 4). The level of utilization of grasses was similar to knapweed, however, native forbs experienced greatest levels of utilization. It is apparent that knapweed was readily grazed because it dominated the herbage removed in each season (Table 4). This resulted from an abundance of knapweed in the grazed area and ready acceptance of knapweed as forage.

### Summary

These preliminary results support the theory that livestock grazing can be used to control spotted knapweed. Knapweed has relatively good forage value and is readily consumed by sheep throughout the year. When knapweed is defoliated, the decline in reproductive output and the slower increase in basal area observed in individual plant trials suggest that grazing can have a negative impact on spotted knapweed. Equally encouraging was the lack of a significant impact of defoliation on the surrounding vegetation. Seedhead production was significantly reduced when defoliation occurred in the flowering phenological stage during individual plant studies. However, grazing during the rosette stage appeared to more

effectively reduce flower production in the grazing trial. Therefore, more information is needed to determine the most effective phenological stage to graze spotted knapweed. Plant responses to defoliation and competition are probably affected by the changes in the yearly environmental conditions; therefore, as the study continues we will have a better grasp of the most effective time and method to graze spotted knapweed.

Table 4. Utilization of a spotted knapweed, other forbs, and grasses on sagebrush steppe rangeland in southeastern Idaho grazed by sheep in 2000.

|               | Grazing Season based on Phenological Stage    |      |                      |      |                      |      |
|---------------|---|------|----------------------|------|----------------------|------|
|               | Rosette                                       |      | Bolting              |      | Flowering            |      |
|               | <u>Stocking Rate</u>                          |      | <u>Stocking Rate</u> |      | <u>Stocking Rate</u> |      |
|               | High  | Low  | High                 | Low  | High                 | Low  |
|               | -----% Utilization -----                      |      |                      |      |                      |      |
| Knapweed      | 72.3  | 57.3 | 53.3                 | 42.2 | 46.6                 | 60.9 |
| Other Forbs   | 86.6  | 73.8 | 90.8                 | 79.0 | 81.1                 | 78.9 |
| Grasses       | 70.3  | 52.4 | 50.1                 | 44.2 | 70.5                 | 58.2 |
| Total Herbage | 74.1  | 59.3 | 56.0                 | 46.7 | 56.7                 | 61.4 |
|               | ----- Proportion of Herbage Removed (%) ----- |      |                      |      |                      |      |
| Knapweed      | 49.8  | 51.8 | 58.9                 | 60.3 | 47.4                 | 63.0 |
| Other Forbs   | 19.8  | 18.7 | 15.7                 | 17.3 | 12.0                 | 8.6  |
| Grasses       | 30.4  | 29.2 | 25.4                 | 22.4 | 40.6                 | 28.4 |

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## On the taxonomy of spotted knapweed (*Centaurea stoebe* L.)

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### **Abstract**

Spotted knapweed was introduced into North America as a seed contaminant from south-eastern Europe in the middle of the 19th century. Today it is a well-known noxious weed in USA and Canada causing economic problems by infesting farm land.

Native to western, central, and eastern Europe spotted knapweed consists of a group of closely related taxa. For the taxonomy and nomenclature various different concepts have been used by different authors resulting in great confusion.

During recent studies the delimitation of the different taxa of spotted knapweed was investigated by using morphological and molecular techniques. According to this work *Centaurea maculosa* LAM. (described from Central France), as well as *C. rhenana* BOREAU, are synonyms of *Centaurea stoebe* L. subsp. *stoebe*, which is native to western and central Europe only. These plants are biennial, strictly monocarpic and diploid ( $2n = 18$ ). All the North American plants called "*Centaurea maculosa*" are perennial, polycarpic and tetraploid ( $2n = 36$ ) and thus must belong to a different taxon. Parallel to the introduction into North America similar plants spread all over Europe. Molecular data confirm that the plants introduced into North America and into Europe belong to the same taxon. Their correct name is *Centaurea stoebe* L. subsp. *micranthos* (GUGLER) HAYEK (synonyms are "*C. biebersteinii*" and "*C. micranthos*").

Keywords: *Centaurea stoebe* L., taxonomy, nomenclature, morphology, distribution

### **Introduction**

Comparing descriptions of spotted knapweed from American literature on biological control and European floras, there are obvious discrepancies in the descriptions of European and American "*C. maculosa*" (i.e. chromosome numbers or life form).

The main reason for this may be the great confusion in taxonomy and nomenclature of spotted knapweed. Even in Europe, where the plants are native, no consensus could be reached in the past 100 years on the number and rank of taxa or the accepted names for them. Besides the taxonomic problems identification of the different taxa was very difficult due to the use of unsuitable characters and high morphological variation.

In a research project funded by the DFG (Deutsche Forschungsgemeinschaft) extensive morphological studies were combined with the use of molecular markers (RAPDs, sequencing of ITS and parts of IGS of nuclear DNA) to re-evaluate the morphological characters used in taxonomy (OCHSMANN 2000).

### **Materials**

As a base for the morphological investigations ca 1000 herbarium specimens were collected from 200 knapweed populations from all over Europe. In addition about 3500 herbarium specimens (including types) from B, BP, G, GOET, JE, LY, M, MICH, MO, P, TSB, W and WU (abbreviations according to HOLMGREN et al. 1990) including material from Europe and North America were studied.

Chromosome counts and molecular analyses were carried out to evaluate the morphological data, but are not reported here (OCHSMANN 1999, OCHSMANN 2000). The sequence data are available in the EMBL database, too.

## Results and Discussion

### Evaluation of Characters Used in Identification

All non-molecular characters of spotted knapweed available were tested for variation and usability for subspecies identification. Characters not listed below showed no variation between the subspecies.

**Branching Pattern and Plant Height** – The degree and pattern of branching as well as the height of the plants show great variation already within populations, apparently due to water and nutrient supply and light conditions (see WAGENITZ 1972): Branching can commence close to the soil surface or in the upper third of the plant only, effecting the number of branches of higher order and the plant habit.

**Width of Capitula** – Statistically there is a difference in capitula width between subsp. *stoebe* and subsp. *micranthos* (table 1). However, the minimum and maximum values of both subspecies are nearly the same so that it becomes quite obvious why the identification by morphological characters is so difficult.

**Table 1 Capitula Width in *Centaurea stoebe* L.**

| taxon                    | mean $\pm$ $\delta_{n-1}$ [mm] | max. [mm] | min. [mm] | n   |
|--------------------------|--------------------------------|-----------|-----------|-----|
| <i>C. stoebe</i> (s.l.)  | 7.7 $\pm$ 1.23                 | 11.5      | 5.0       | 348 |
| subsp. <i>stoebe</i>     | 8.5 $\pm$ 0.96                 | 11.0      | 6.5       | 108 |
| subsp. <i>micranthos</i> | 6.7 $\pm$ 0.87                 | 8.0       | 5.0       | 100 |

**Phyllary Appendages** – The number of veins and the colour of the cilia of the phyllaries already varies within each capitulum and is not suitable for identification. However, the number of lateral cilia (table 2) can be used to separate subsp. *stoebe* and subsp. *micranthos* (HEGI 1928). To use this character, phyllaries from the middle of fully developed capitula of normal plants have to be used. Capitula from late flowering plants or branches can show extreme differences in size, colour and number of cilia.

**Table 2 Number of Lateral Cilia in *Centaurea stoebe* L.**

| subspecies               | mean number of cilia per side |
|--------------------------|-------------------------------|
| subsp. <i>stoebe</i>     | 6-10                          |
| subsp. <i>micranthos</i> | 4-7                           |

**Pappus Length** – The taxonomic value of pappus length has been overestimated in the literature by several authors, especially by HAYEK (1901). Though it had been recognized by GUGLER (1907) or STOJANOFF & ACHTAROFF (1935), its high variation was not taken into account. Like in the capitula width there is a slight difference in mean pappus length between subsp. *stoebe* and subsp. *micranthos* but no difference in the absolute range (table 3).



**Table 3 Pappus Length in *Centaurea stoebe* L.**

| taxon                    | mean $\pm$ $\delta_{n-1}$ [mm] | max. [mm] | min. [mm] | n   |
|--------------------------|--------------------------------|-----------|-----------|-----|
| <i>C. stoebe</i> (s.l.)  | 1.2 $\pm$ 0.43                 | 2.5       | 0         | 264 |
| subsp. <i>stoebe</i>     | 1.5 $\pm$ 0.40                 | 2.5       | 0.1       | 79  |
| subsp. <i>micranthos</i> | 1.0 $\pm$ 0.36                 | 2.5       | 0.2       | 79  |

**Chromosome Number (Ploidy Level)** – Chromosome number turned out to be the only unequivocal character for the delimitation of subsp. *stoebe* and subsp. *micranthos*: subsp. *stoebe* is diploid with  $2n = 18$  and subsp. *micranthos* is tetraploid with  $2n = 36$ . However, the determination of chromosome numbers is expendable and restricted to living material (at least living achenes).

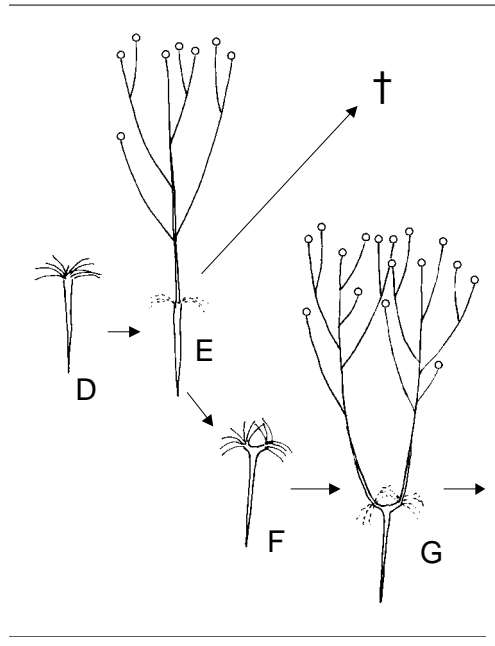
**Life Form and Number of Stems** – The life form is correlated with the number of stems and also with the ploidy level (see above). While the monocarpic subsp. *stoebe* normally develops a single stem from a single rosette, the polycarpic subsp. *micranthos* starts with a single stem in its first flowering period, adding additional rosettes in late summer. In the following year these rosettes develop one stem each and so on (Fig. 1). The problem is that young plants of subsp. *micranthos* having only one stem closely resemble plants of subsp. *stoebe* at the time of flowering, the difference becoming obvious only afterwards when the new rosettes from axillary buds appear. Unfortunately, there are still cases where the subspecies cannot be determined due to the young age of the plants or the quality of the material.

**Ecology** – *C. stoebe* subsp. *stoebe* and subsp. *micranthos* both prefer sun-exposed, dry habitats, but show no preference for any particular soil type. They can be found on sand, slate, granite, limestone as well as serpentine. However, due to their life forms subsp. *stoebe* and subsp. *micranthos* show clear differences in competition with other plants. The monocarpic subsp. *stoebe* cannot compete with perennial species so that it is found only in habitats with open ground where the seedlings can establish themselves. It is mainly growing in natural stands on xerothermic habitats (e.g. dry meadows) or on rocks. On the contrary the perennial subsp. *micranthos* can tolerate dense vegetation once the plants are established. This is the reason why only this subspecies can be invasive.

### **Taxonomy**

The recognition of the dependence of life form and ploidy level and its correlation with the subspecies is the key for solving the taxonomic problem of subsp. *stoebe* and subsp. *micranthos*. Even HAYEK (1901) did not realize this correlation. In his work he lists biennial and perennial plants for subsp. *stoebe* and subsp. *micranthos*, respectively. As his work was the base for many later treatments of *C. stoebe* s.l. the results of these works must be considered with care (especially chromosome counts and distribution data). For full synonymy and publication data see OCHSMANN (2000).

**Fig. 1** Life Form and Number of Stems of *Centaurea stoebe* L. (D → E → †: monocarpic



plant, D → E → F → G → ... : polycarpic plant).

***Centaurea stoebe* L.**

*Type* – Not designated. A neo-typification is in preparation (OCHSMANN & WAGENITZ, in prep.) because no authentic material exists in the herbaria used by LINNAEUS (JARVIS 1996, in litt.).

*Selected Synonyms* – *C. biebersteinii* DC.; *C. maculosa* LAM. subsp. *biebersteinii* (DC.) NYMAN; *C. stoebe* L. subsp. *micranthos* (GUGLER) HAYEK var. *biebersteinii* (DC.) STOJ. & ACHT.; *C. savranica* KLOKOV; *C. rhenana* BOREAU subsp. *savranica* (KLOKOV) DOSTÁL; *C. pseudomaculosa* DOBROČZ.; *C. rhenana* BOREAU subsp. *pseudomaculosa* (DOBROČZ.) DOSTÁL; *C. paniculata* auct. non L.: JACQ., BESSER, KOCH.

*Remarks* – Apart from the imprecise diagnosis in *Species plantarum* (LINNAEUS 1753) no authentic material exists in the herbaria used by LINNAEUS (LINN; S-LINN; Herb. BURSER, UPS; Herb. ROYEN, L). CLUSIUS (1601) and the other sources cited by LINNAEUS (BAUHIN 1623, ROYEN 1740) do not allow an unequivocal identification. Problems start with the delimitation of *C. stoebe* from *C. paniculata*, also described by LINNAEUS (1753). For the geographic distribution LINNAEUS (1753: 914) lists Austria and Siberia in addition to France and Spain. If the indication for Austria and Siberia by LINNAEUS is taken as correct then *C. stoebe* may be considered part of *C. paniculata*, as done by several authors (FIORI 1904a, 1904b; ARÈNES 1951; EHRENDORFER 1973; BINZ & HEITZ 1990). This results in an even more complicated *C. paniculata* aggregate which could include many species of sect. *Acrolophus* from Spain to Siberia.

A much better solution was suggested by LAMARCK (1785), who recognised the clear morphological distinction between the western and central to eastern plants, despite of LINNAEUS' geographic specification. LAMARCK restricted the use of *C. paniculata* to the western plants occurring in Spain and France only. For the rest he used *C. stoebe*. This interpretation is strongly supported by molecular and ecological data (OCHSMANN 2000).

Another effect of this solution is that in either group the oldest name is used (see SCHINZ & THELLUNG 1909; NEUMAYER 1942).

The problems described above were the reason why other names instead of *C. stoebe* were used, mainly *C. maculosa* and *C. rhenana*, but this did not solve the problem. *C. stoebe*, *C. maculosa* and *C. rhenana* were used by different authors in almost every possible combination with various delimitations. Apart from that, as both *C. maculosa* and *C. rhenana* originally applied only to plants from small areas of France, the oldest name should be used for the whole geographic area. This opinion is shared by recent treatments for central Europe (WAGENITZ 1987), Germany (LANGE 1996, OCHSMANN 1998) or Switzerland (AESCHIMANN & HEITZ 1996).

***Centaurea stoebe* L. subsp. *stoebe***

*Selected Synonyms* – *C. maculosa* LAM.; *C. paniculata* L. subsp. *maculosa* (LAM.) BRIQ.; *C. paniculata* L. var. *maculosa* (LAM.); *C. stoebe* L. subsp. *maculosa* (LAM.) SCHINZ & THELL; *C. paniculata* L. subsp. *maculosa* (LAM.) BRIQ. var. *maculosa* BRIQ.; *C. maculosa* LAM. var. *albida* LECOQ & LAMOTTE; *C. maculosa* LAM. subsp. *albida* (LECOQ & LAMOTTE); *C. muretii* JORD ("mureti"); *C. coerulescens* WILLD. subsp. *muretii* (JORD.) NYMAN; *C. maculosa* LAM. var. *muretii* (JORD.) SCHINZ & KELLER; *C. maculosa* LAM. f. *muretii* (JORD.) GUGLER; *C. maculosa* LAM. subsp. *muretii* (JORD.) JANCHEN ex H.P. FUCHS-ECKERT; *C. pedemontana* JORD; *C. subalbida* JORD.; *C. paniculata* L. subsp. *leucophaea* (JORD.) BRIQ. var. *subalbida* (JORD.) ROUY; *C. paniculata* L. subsp. *maculosa* var. *subalbida* (JORD.) BRIQ. & CAVILL.; *C. maculosa* LAM. subsp. *subalbida* (JORD.) DOSTÁL; *C. tenuisecta* JORD.; *C. paniculata* L. subsp. *maculosa* (LAM.) BRIQ. var. *tenuisecta* (JORD.) ROUY; *C. chaubardii* RCHB. f. "*chaubardi*"; *C. paniculata* L. subsp. *maculosa* var. *maculosa* subvar. *chaubardii* (RCHB. f.) ARENES ("var. *eumaculosa*"); *C. maculosa* LAM. subsp. *chaubardii* (RCHB. f.) DOSTAL; *C. rhenana* BOREAU; *C. paniculata* L. var. *maculosa* (LAM.) FIORI f. *rhenana* (BOREAU) FIORI; *C. paniculata* L. subsp. *maculosa* (LAM.) BRIQ. var. *rhenana* ROUY; *C. maculosa* LAM. subsp. *rhenana* (BOREAU) GUGLER; *C. stoebe* L. subsp. *rhenana* (BOREAU) SCHINZ & THELL.; *C. paniculata* auct. non L. p.p.: W.D.J. KOCH.

*Description* – Biennial to perennial, monocarpic; usually single-stemmed, erect, ca (10-) 20-120 cm high; stem usually paniculate branched in the upper half only, with relatively short branches; plants weakly woolly; rosette leaves one- to many-times pinnatifid to pinnatisect with narrow segments, upper leaves mostly undivided; capitula single at the ends of the branches, 6.5-11 mm wide, ovate to U-shaped; phyllary appendages dark brown to black, triangular, acute, ciliate, with 6-10 cilia per side, each 1-2 mm long; flowers purple, ray florets present, flowering VI-X; achenes ca 3-4 mm long; pappus ca 1-2.5 mm, rarely shorter.

*Chromosome number* –  $2n = 18, 18 + 2B$  (see OCHSMANN 2000 for references)

*Natural distribution* – In western, central and eastern Europe (A, BY, CH, CZ, D, F, H, HR, I, LT, LV, MD, PL, RO, RUS, SK, UA; see Fig. 2) on dry, sun-exposed habitats or rocks with low vegetation cover, up to 1,500 m above sea level.

*Remarks* – Due to the high variation of most morphological characters formerly used for identification a separation of *C. maculosa* LAM, *C. rhenana* BOREAU and *C. stoebe* L. ssp. *stoebe* is impossible. LAMARCK's (1785: 669) diagnosis does not provide any diagnostic character, on the contrary: he does not seem to have seen any material of the LINNEAN species. Neither "v.s." [vidi siccum] nor "v.v." [vidi vivum] are mentioned at the end of LAMARCK's diagnosis of *C. maculosa* and his indication of the distribution of *C. stoebe* appears to be a mere translation of the LINNEAN text. Though BOREAU (1857: 355) gives

some differential characters against *C. maculosa* for his *C. rhenana*, at the same time he refers to REICHENBACH's figure (1852: tab. 48) of *C. maculosa* for his own species.

***Centaurea stoebe* L. subsp. *micranthos* (GUGLER) HAYEK**

Type – "C. BAENITZ, Herb. europ. sine No. [...]", see GUGLER (1907: 169) (BP, Isotype? M)

Basionym – *C. maculosa* LAM. subsp. *micranthos* GUGLER

Selected Synonyms – *C. paniculata* L. var. *micranthos* GRISEB.; *C. australis* PANČIĆ ex A. KERN.; *C. sublanata* (DC.) BOISS. subsp. *australis* (PANČIĆ) NYMAN; *C. stoebe* L. subsp. *micranthos* (GUGLER) HAYEK var. *australis* (PANČIĆ) HAYEK; *C. biebersteinii* DC. subsp. *australis* (PANČIĆ) DOSTÁL; *C. radoslavoffii* URUM.; *C. stoebe* L. subsp. *micranthos* (GUGLER) HAYEK var. *radoslavoffii* (URUM.) HAYEK; *C. stoebe* L. subsp. *micranthos* (GUGLER) HAYEK var. *australis* (PANČIĆ) STOJ. & ACHT. f. *radoslavoffii* (URUM.) STOJ. & ACHT.; *C. biebersteinii* DC. subsp. *radoslavoffii* (URUM.) DOSTÁL; *C. maculosa* LAM. subsp. *micranthos* f. *rhodopaea* HAYEK & J. WAGNER; *C. maculosa* LAM. subsp. *micranthos* (GUGLER) HAYEK f. *rhodopaea* (HAYEK & J. WAGNER) HAYEK; *C. stoebe* L. subsp. *micranthos* (GUGLER) HAYEK var. *australis* (PANČIĆ) STOJ. & ACHT. f. *rhodopaea* (HAYEK & J. WAGNER) STOJ. & ACHT.; *C. biebersteinii* DC. subsp. *rhodopaea* (HAYEK & J. WAGNER) DOSTÁL; *C. biebersteinii* auct. non DC.: NYMAN; *C. maculosa* auct. american. non LAM.; *C. paniculata* auct. non L.: M. BIEB.

Description – **Perennial** (according to BOGGS & STORY (1987) up to 9 years), **polycarpic; usually many-stemmed**, erect, ca 40-150 (-200) cm high; stem paniculate branched, with relatively long branches; plants lanate; rosette leaves one- to many-times pinnatifid to pinnatisect with narrow sections, upper leaves mostly undivided; capitula single at the ends of the branches, 5-8 mm wide, narrow ovate to ovate; phyllary appendages often tinged with dark violet, sometimes ± lanate, black, triangular, acute, ciliate, with 4-7 cilia per side, each 1-2 mm long; flowers purple, ray florets present, flowering VI-IX; achenes ca 3-3.5 (-4) mm long; pappus ca (0.2-) 1-2.5 mm.

Chromosome number –  $2n = 36$  (References for  $2n = 18$  may be based on misidentifications; see OCHSMANN 2000 for literature).

Distribution – Natural from southern central to south-eastern Europe and northwestern Asia (parts of A, BG, EST, H, HR, LT, LV, MD, MK, RO, RUS, SLO, UA, YU), but introduced into almost all parts of Europe, including southern Sweden and into North America (USA, Canada). Growing on sand dunes, ruderal places, railroad and port areas, road sides, grassland, in the USA up to more than 2.600 m above sea level.

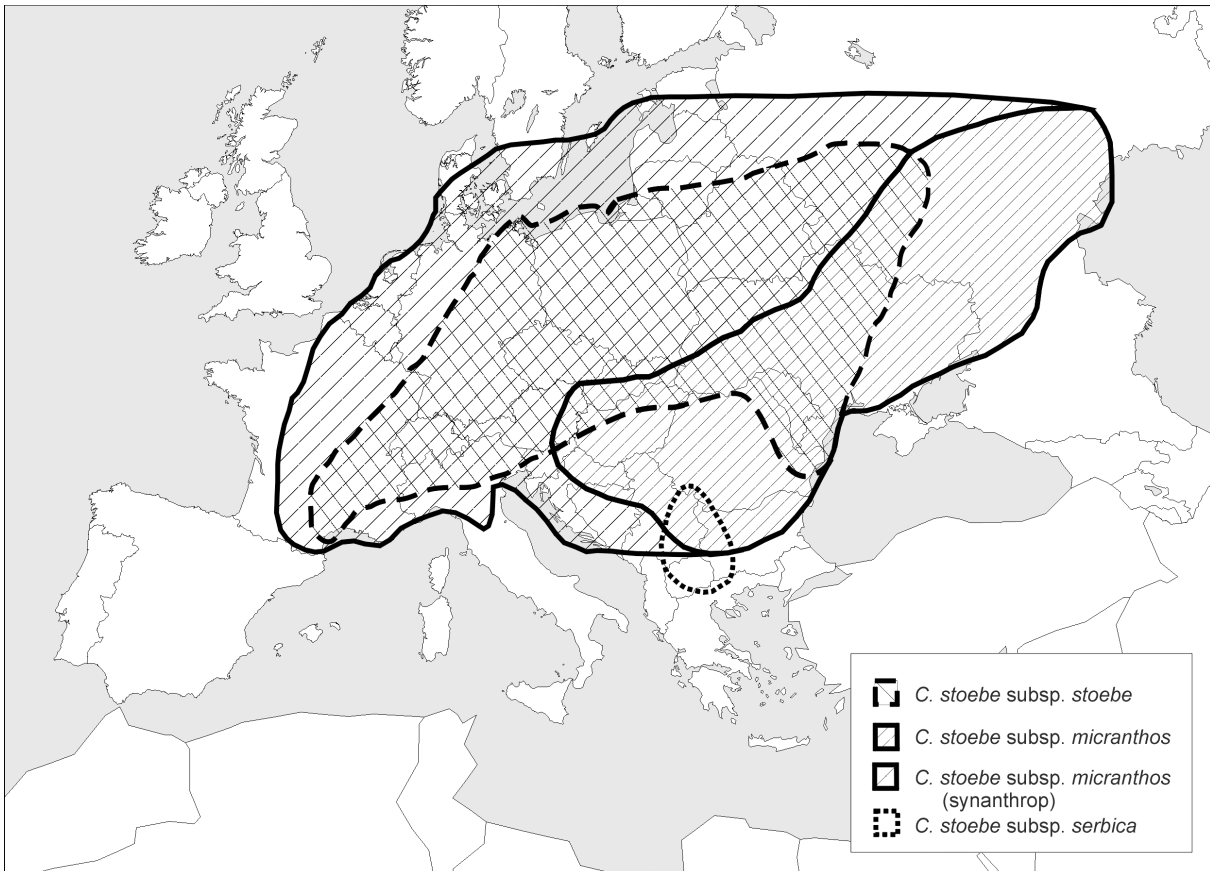
Remarks – GMELIN's (1770: 135, tab. 23) *Centaurea micranthos* is not validly published because both of the synonyms are mentioned with a "?" and a description or a figure showing details are lacking. The name *C. micranthos* S.G. GMEL. ex HAYEK 1901 is illegitimate because of *C. micrantha* HOFFMANNS. & LINK 1820. Though listed as a synonym by HAYEK and many others *C. biebersteinii* DC. does not belong to this taxon. According to the type material *C. biebersteinii* DC. (in G-DC) is closely related to subsp. *micranthos*, but because of its much broader capitula and the monocarpic life form it cannot be identical with this taxon.

## Conclusions

The morphological, ecological and chromosome data clearly indicate that the American plants of spotted knapweed belong to the tetraploid subsp. *micranthos* and not to subsp. *stoebe*.

In fact subsp. *micranthos* has been a very successful neophyte not only in North America but also in most parts of Europe (Fig. 2) in the past 100 to 150 years. Like in North America it was mainly dispersed by railway and ship transport of crops. In Europe it presumably has doubled its area of distribution (Fig. 2), but here it is mostly restricted to ruderal habitats such as ports, railway tracks and industrial sites. In central and western Europe it does not infest farmland, possibly due to the climate and herbivorous insects. Due to the lack of economic importance, the occurrence of native populations and the difficulties in determination the introduced plants of subsp. *micranthos* have been overlooked in many cases.

Even when the taxonomic view of the author is not accepted and *Centaurea maculosa* LAM. is used instead of *C. stoebe* L. for spotted knapweed, it is important to recognize that different (infraspecific) taxa of spotted knapweed exist and that only one of them (subsp. *micranthos*) was introduced to North America. Only with knowledge of the two subspecies of *C. stoebe* it is possible to understand the biology and ecology of spotted knapweed and to improve the methods of control in North America.



**Fig. 2** Distribution of the subspecies of *Centaurea stoebe* L.

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## Yellow Starthistle

### **Examination of a Toxin Produced by *Alternaria* spp. on Yellow Starthistle**

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#### **Abstract**

Several *Alternaria* spp. were isolated from leaf tissue of *Centaurea solstitialis*, yellow starthistle (YST), in southern France. Certain fungal pathogens, especially in the genus *Alternaria*, produce low-molecular-weight compounds known as host-selective toxins. Attempts to induce necrotic symptoms on YST tissue with spores of the isolated *Alternaria* spp. have failed to date. However, the effect of the culture filtrates produced by these species was examined. Several isolated *Alternaria* spp. were grown stationary in the dark for 6 weeks in a defined liquid medium. A cell-free filtrate was obtained by passing through a 0.2 µm filter. A portion of this filtrate was autoclaved. Drops of the autoclaved and nonautoclaved filtrates were placed on detached YST leaves of various ages. After 96 hours, small necrotic lesions developed on the point of contact with the filtrates from the isolates, but not the controls. Three-week-old YST green callus pieces were placed in sterile wells containing liquid MS medium (control), 100, 50, 25, 10 or 1% of the filtrates (diluted with liquid MS medium). After 12 hours at 25°C, calli exposed to the 100% filtrates from the isolates turned brown. After 96 hours, all calli turned brown, except for the control and the 1% treatment. These results indicate the likely production of a toxin by the *Alternaria* spp. against YST.

Key words: Yellow starthistle, *Centaurea solstitialis*, biological control, *Alternaria*

#### **Introduction**

Infestations of the noxious weed yellow starthistle (*Centaurea solstitialis* L.) are reported in 23 of the 48 contiguous states but are heaviest in the western United States (Maddox et al., 1985). Yellow starthistle is considered a weed because it displaces desirable plants and consumption by horses can be fatal. It is a prime target for classical biological control because conventional control strategies have been inadequate due to the size of infestations and the economic and environmental costs of chemical control. Six exotic insect species have been introduced but with limited success to sufficiently restore land to its original habitat (Balciunas and Villegas, 1999; Turner et al., 1995). A few diseases on yellow starthistle in California have been described but they have not had a significant impact (Klisiewicz, 1986; Pitcairn et al., 1999; Woods and Fogle, 1998). The origins of yellow starthistle are believed to be in the Mediterranean region of Eurasia. The search for pathogenic fungi in the region of origin has resulted in the isolation of several *Alternaria* spp. from leaf tissue.

*Alternaria* spp. are known throughout the world causing many diseases on plants of economic importance (Rotem, 1994). Some of these *Alternaria* spp. have been found to be fairly host specific (Walker, 1982). Symptoms on plants caused by infection from *Alternaria* spp. may be leaf spots, necrosis, and possible plant death (Rotem, 1994).

Certain fungal pathogens, especially in the genus *Alternaria*, produce low-molecular-weight compounds known as host-specific toxins that determine their host range and



contribute to their virulence or pathogenicity (Otani et al., 1995). The first host-specific toxicity in culture filtrates of *Alternaria alternata* (Fr.) Keissler was found by Tanaka (1933). Since then, there have been other toxins discovered from *A. alternata* (Akamatsu et al., 1997; Bobylev et al., 1996). One such toxin has been found to be specific on a relative of yellow starthistle, spotted knapweed (*Centaurea maculosa* L.; Bobylev et al., 1996; Strobel et al., 1990).

We hope that by studying these toxins, novel compounds can be discovered to help control invasive weeds. To help in this purpose, a quick bioassay was developed involving callus tissue of yellow starthistle. It was the purpose of this study to determine if *Alternaria* spp. isolated from the weed produce metabolites that may be toxic to tissue of yellow starthistle.

## Materials and Methods

**Fungal culture.** Isolation of *Alternaria* spp. was conducted by collecting yellow starthistle tissue that exhibited minor necrotic symptoms at the rosette stage in southern France. The tissue was surface-sterilized in 0.5% sodium hypochlorite for 1 min and rinsed twice in sterile distilled water. The tissue was blotted dry and transferred to petri plates containing solidified water agar with 50 ppm streptomycin sulfate (WA+S). The plates were incubated under light at 20 C. Once mycelium was observed growing from the tissue, a small agar plug containing the mycelium was transferred to solidified half-strength potato dextrose agar (1/2PDA). These plates were placed under continuous light in an incubator at 20 C. The fungal isolates were identified to the genus *Alternaria* by their characteristic conidia. Three isolates were collected and labelled YST-3, YST-4, and YST-6. Cultures were maintained on 1/2PDA at 20 C for further use.

**Plant Inoculations.** Seeds of yellow starthistle were germinated on moistened filter paper. After 5 d, the seedlings were transferred to potting mix and allowed to establish for 5 d. Those seedlings which had developed true secondary leaves were transferred to small plastic pots (100-cm<sup>3</sup> volume) with 5 seedlings per pot. Conidia of the tested *Alternaria* spp. were produced by a slight modification of the method by Shahin and Shepard (1979). Cultures were grown on 1/2PDA for 7 d at 20 C. Mycelial plugs (5-mm diameter) were removed from the edge of the actively growing culture and transferred to plates containing the sporulation medium (S-M). The S-M was composed of 20 g sucrose, 30 g CaCO<sub>3</sub>, and 20 g of agar per liter of water. The pH was adjusted to 7.4 with 1 M HCl before autoclaving. Sterile distilled water (3 ml) was added to the plates to partially cover the mycelial blocks and the plates were incubated at 20 C in the dark. After 5 d, distilled water plus 0.25% (v/v) silicone-polyether copolymer (Silwet L-77; Loveland Industries, Inc., Greeley, CO) was poured over the medium and gently swirled to dislodge the conidia. The resulting suspension was poured into a sterile beaker and the plates were washed once more following the same procedure. The spores were counted with a haemocytometer. Conidia of the *Alternaria* spp. were adjusted to 5 X 10<sup>6</sup> conidia ml<sup>-1</sup>. The yellow starthistle seedlings were sprayed until run-off with the conidial suspensions or a control of water plus 0.25% Silwet. The cups were placed in closed plastic bags and placed in a growth chamber with 16-h daylight at a continuous temperature of 24 C. The plants were observed over time and removed from the plastic bags after 4 d.

**Callus production.** A modified half-strength Murashige and Skoogs (1/2MS) medium was prepared as described (Murashige and Skoog, 1962). The modified stock solution of macro-elements consisted of 825 mg NH<sub>4</sub>NO<sub>3</sub>, 950 mg KNO<sub>3</sub>, 220 mg CaCl<sub>2</sub>-2H<sub>2</sub>O, 185 mg MgSO<sub>4</sub>-7H<sub>2</sub>O, and 85 mg KH<sub>2</sub>PO<sub>4</sub> in 1 liter of distilled water. A stock solution of micro-elements was prepared by adding 0.83 mg KI, 6.2 mg H<sub>3</sub>BO<sub>3</sub>, 16.9 mg MnSO<sub>4</sub>-H<sub>2</sub>O, 8.6 mg

ZnSO<sub>4</sub>-7 H<sub>2</sub>O, 0.25 mg Na<sub>2</sub>MoO<sub>4</sub>-2 H<sub>2</sub>O, 0.025 mg CuSO<sub>4</sub>-5 H<sub>2</sub>O, and 0.025 mg CoCl<sub>2</sub>-6 H<sub>2</sub>O to 1 liter of distilled water. Murashige and Skoog vitamin mixture was purchased and prepared to make a 1000X stock solution (Duchefa, Haarlem, The Netherlands). The medium was prepared by combining 50 ml of the macro-elements stock solution, 5 ml of the micro-elements stock solution, 1 ml of the vitamin mixture stock solution, 36.7 mg of FeNa-EDTA, and 30 g of sucrose to distilled water for a final volume of 1 liter. The pH was adjusted to 6.0 and 8 g of phyto agar (Duchefa) was added. The medium was autoclaved and dispensed into sterile baby food jars. Young rosette leaves (approximately 1-month-old) of yellow starthistle were removed at the base of the plant. The leaf sections were surface sterilized for 30 seconds in 75% ethanol plus 2 or 3 drops of Tween 20 (Sigma Chemicals, St. Louis, MO). The leaves were transferred to 0.5% calcium hypochlorite. After 20 min, the leaves were rinsed for 10 min in sterile distilled water and another two times for 5 min in sterile distilled water. The sections were placed into baby food jars containing 1/2MS medium. The jars were placed in an incubator at 26 C with 16 h of fluorescent light. After callus tissue formed, the calli were broken off and transferred to fresh 1/2MS medium. Calli were transferred every 3 weeks.

**Culture filtrate production and testing.** Culture filtrate medium was produced as described by Maiero et al. (1991) and contained 1 g KH<sub>2</sub>PO<sub>4</sub>, 0.5 g MgSO<sub>4</sub>, 6 g casein hydrolysate, 100 g sucrose, 1 mg FeSO<sub>4</sub>, 0.15 mg CuSO<sub>4</sub>, 0.1 mg ZnSO<sub>4</sub>, and 0.1 mg Na<sub>2</sub>MoO<sub>4</sub> per liter of distilled water. The medium was adjusted to pH 4.9 with 0.1 M HCl and autoclaved. Five mycelial plugs (2-mm diameter) from the edge of an actively growing colony of the tested *Alternaria* spp. were added to 100 ml of the cooled medium. The cultures were incubated at room temperature in the dark for 6 weeks under stationary conditions. Culture filtrates were filtered by pouring the medium through several layers of cheesecloth. The filtrate was sterilized by passing through a 0.2 µm filter and stored at 4 C. Ten milliliters of the culture filtrate was placed in a culture tube and placed in boiling water for 20 min. Rosette leaves of yellow starthistle were removed from the plant and placed in a petri plate containing moistened filter paper. A drop of the culture filtrate was placed on each of the leaves, including a water control. The plates were placed in a growth chamber at 24 C. The presence of necrotic symptoms was noted over time.

Those isolates which produced necrotic symptoms were rated as positive and tested further. Half-strength liquid MS medium was produced as described above, except without the added agar. After sterilization, the liquid medium was pipetted into a sterilized 24-well microtiter plate (Greiner Labortechnik, Frickenhausen, Germany) containing 0, 1, 0.5, 0.25, 0.1, and 0.01 ml of the culture filtrate for a final volume of 1 ml. This corresponds to a control of no culture filtrate and dilutions of 0, 50, 75, 90, and 99%, respectively. Green healthy calli, approximately 1-cm diameter were selected and placed in each of the wells (2 replicates per dilution). The plate was placed in a 26 C incubator with 16 h of fluorescent light. Every 24 h for 96 h, the appearance of the calli were rated on a 1 to 5 scale with a rating of one meaning healthy green calli, a rating of two meaning slight discoloration, a rating of three showing obvious brown discoloration, a four meaning complete dark brown discoloration, and a five indicating tissue degradation occurred. The experiment was conducted a total of three times for each of the *Alternaria* spp. culture filtrates. Data were subjected to analysis of variance using GLM (SAS Institute). The means were compared to the control statistically using least significant differences.

## Results and Discussion

Inoculations with the conidia of the *Alternaria* spp. isolates showed very few disease symptoms. Out of the 25 plants inoculated with isolate YST-4, only one seedling died and four others showed minor symptoms on the cotyledons. Over time these four plants fully recovered. Inoculations with YST-6 did not show any symptoms on any of the seedlings. Conidia of isolate YST-3 could not be produced and, therefore, could not be tested. Although the majority of the plants recovered from inoculations with YST-4, there was some evidence of damage. Fungi like the *Alternaria* spp. tested, although not likely of widespread mortality, might inhibit the defense mechanisms of yellow starthistle and could be used in conjunction with some other pathogens or control agents (Scheffer and Livingston, 1984).

However, the culture filtrates of the *Alternaria* spp. isolates tested showed necrotic lesions on detached leaves of YST regardless if the filtrates were boiled or not. This suggests that the toxin is not a protein or enzyme. This was also the observation by Mehta and Brogin (2000) of a toxin produced by *Stemphylium solani*. When the YST-3, YST-4, and YST-6 culture filtrates were applied directly to the leaves of young YST seedlings the leaves became dried and with some mortality. These plants did not recover as well as the conidia-treated seedlings.

Callus tissue could easily be produced by the indicated procedure when young leaf tissue was utilized. This gives the possibility of developing a bioassay to quickly determine if a solution is toxic to yellow starthistle tissue. This technique was utilized previously by Souissi and Kremer (1998) to screen isolated microorganisms on leafy spurge (*Euphorbia esula* L.). Even though yellow starthistle whole plant regeneration was very rare from callus, it did occasionally occur. It was observed that these whole plants were produced from calli that were derived from the bases of young leaves. Further studies need to be continued to follow this observation.

Testing the culture filtrates on calli of yellow starthistle showed positive results. All three of the culture filtrates tested at full strength showed an immediate impact within 24 h. Isolates YST-3, YST-4, and YST-6 had ratings of 3.2, 3.5, and 3.5, respectively. This was significantly different than the control which had a rating of 1.0 ( $P \leq 0.05$ ). For isolates YST-3 and YST-4 dilutions of 50, 75, 90, and 99% were not different than the control at 24 h (data not shown). However, dilutions of isolate YST-6 at 50 and 75% had ratings of 3.5 and 2.8, which were significantly different. After 96 h, the treatments of full strength, 50, 75, 90, and 99% dilutions of isolate YST-4 had ratings of 4.5, 4.0, 3.3, 2.7, and 1.3, respectively. Isolate YST-3 had ratings of 4.2, 4.2, 3.3, 3.0, and 1.2, respectively. Isolate YST-6 had ratings of 4.0, 4.0, 3.8, 2.7 and 1.5, respectively. For all isolates after 96 h, only the 99% dilution was not significantly different than the control which had a rating of 1.0 ( $P \leq 0.05$ ).

## Summary

The current study clearly demonstrates that a heat-stable compound is produced by the *Alternaria* spp. that causes cell necrosis, although infection may not be as apparent. A previous study showed that when conidia of *A. cassiae* Jurair and Khan were sprayed on sicklepod, penetration was only observed occasionally, but necrosis extended to cells adjacent to those beneath the appressoria (Van Dyke and Haning, 1983). This would suggest that a diffusible toxin was involved. Future work could involve looking at the histology of the yellow starthistle-specific host-pathogen interactions. Further work is also needed to determine the chemical structure of the toxin(s) and whether the toxin(s) is/are host-specific. The use of the callus bioassay was a very quick and easy way to determine if the filtrate had an effect on yellow starthistle tissue. By studying these produced toxins, a better insight into

development of novel compounds for weed control can be developed and their impact on the host-pathogen interaction understood.

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**The Biological Control of Yellow Starthistle in the Western U.S.:  
Four Decades of Progress**

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**Abstract**

Yellow starthistle, *Centaurea solstitialis* L. (Asteraceae), is an Eurasian winter annual that was accidentally introduced into North America in the mid-1800s. This highly invasive, monopolistic weed thrives in disturbed semiarid environments as typified by overgrazed rangelands and pastures, abandoned croplands, wastelands, forests, and transportation rights-of-way. It presently infests in excess of 3.7 million ha in 23 states, with the most impacted states being Arizona, California, Idaho, Oregon, and Washington. A biological control program was initiated in the United States in 1969 with the importation of the capitulum-infesting fly *Urophora jaculata* Rondani. During the 1980s and 1990s, six additional capitulum-feeding dipteran and coleopteran phytophages were intentionally or inadvertently established for the weed's suppression. Of these natural enemies, *Eustenopus villosus* (Boheman) and *Chaetorellia succinea* (Costa) have begun to significantly impact yellow starthistle seed production. Several other bioagents are being evaluated for possible release. Field observations suggest that the unilateral application of biological control agents will not adequately diminish *C. solstitialis* infestations below economic damage levels. Satisfactory yellow starthistle population reductions will only be realized from the utilization of well-planned, coordinated, and properly executed long-term integrated weed management programs in impacted ecosystems.

Key words: yellow starthistle, *Centaurea solstitialis*, biological control, insects, integrated weed management

The genus *Centaurea* (Asteraceae: Cardueae), whose members are collectively referred to as the knapweeds and starthistles, is comprised of over 1,000 species of predominantly Eurasian origin (Roché and Roché 1991). The unintentional introduction into the United States, and subsequent unrestrained spread and naturalization of certain of these aggressive *Centaurea* species during the last 100 years, has negatively impacted crop- and wildland ecosystem health and productivity. Species of note include diffuse knapweed (*C. diffusa* Lam.), meadow knapweed (*C. pratensis* Thuill.), Russian knapweed (*C. = Acroptilon repens* L.), spotted knapweed (*C. maculosa* Lam.), squarrose knapweed (*C. squarrosa* Willd.), purple starthistle (*C. calcitrapa* L.), and yellow starthistle (*C. solstitialis* L.).

Yellow starthistle, also known as St. Barnaby's thistle and golden thistle, has become a weed of extreme importance in 23 states, with the most serious infestations being found in Arizona, California, Idaho, Oregon, and Washington (Maddox et al. 1985). In these states, in excess of 3.7 million ha of rangeland, pastures, abandoned croplands, natural areas, wastelands, and roadsides have been degraded by this nonindigenous plant (Lass et al. 1996). It is believed that the weed was most likely introduced into North America on multiple occasions as a contaminant of alfalfa seed (*Medicago sativa* L.) originating from the Mediterranean Basin of Europe (Roché and Talbott 1986). The plant prefers south-facing sites with deep, well-drained soils that receive less than 50 cm of precipitation annually, but

it also thrives in shallow, rocky soils (Lass et al. 1999) and areas of even higher precipitation (Callihan et al. 1984) The weed continues to expand its naturalized range annually (Talbot 1987).

Yellow starthistle is a herbaceous winter annual. Achene germination is normally initiated by autumn rains but some germination may also occur during the winter and spring. Rapid germination and seedling root growth give yellow starthistle the ability to occupy a site by capturing and utilizing resources before most other competing vegetation. The transition from seedling to rosette begins in late winter and continues into late spring. The plant bolts during May and June to produce erect spreading stems that are branched from the base and grow to a height of 20 to 75 cm. Bud development occurs from mid-June to early July, and anthesis occurs from mid-July to September (Maddox 1981). The bright yellow floral capitula occur singly at the ends of branches, the capitula bracts being armed with long, sharp spines. Several thousand achenes may be produced per plant under optimal conditions (Maddox et al. 1985). Both plumed and plumeless achenes are formed in a capitulum, with nearly three times as many plumed achenes being produced (Joley et al. 1992). Plumed achenes are spread short distances by the wind (Roché 1992); plumeless achenes fall to the soil beneath the parent plant to reinfest the site (Callihan et al. 1984). About 90% of the achenes are viable at maturity, but the remaining percentage becomes dormant and may remain viable in the soil for a decade or more (Callihan et al. 1993).

The dense canopy formed by a monoculture of the weed blocks light penetration to the soil surface and effectively eliminates the emergence or growth of competing, edible forage species, reduces livestock and wildlife grazing capacity, and diminishes endemic plant species diversity. Dried plant skeletons can also provide fuel for late summer wildfires. The capitula spines may physically injure browsing animals and also effectively deter human access to heavily infested recreational or other lands. If ingested in sufficient quantity by horses, the plant can cause a chronic and potentially fatal neurological disorder called "chewing disease", or equine nigropallidal encephalomalacia (Cordy 1978, Panter 1991).

The rapid spread of yellow starthistle has resulted in annual expenditures of private and public funds for management programs. Effective suppression of existing infestations must involve a systematic and persistent effort over several years, the effort being based upon the utilization of multiple, cost-effective population management methods. Yellow starthistle suppression methods include herbicides, mowing, hand-pulling/cultivation, prescribed burning, encouraging or reseeding competitive, perennial forage plant species, controlled grazing, and biological control organisms (Thomsen et al. 1996).

The employment of biotic organisms is an attractive, long-term population reduction technique for yellow starthistle in the western United States because a large percentage of the land dominated by this weed is only marginally productive, and investments required for the application of various chemical, cultural, and mechanical controls are frequently not economically feasible. The successful use of arthropod biological control agents may aid in lessening the plant's invasiveness and abundance, and represents an effective, low cost, and environmentally acceptable form of weed suppression, especially when integrated with other management methods.

Field surveys of arthropods and pathogens associated with yellow starthistle in Eurasia were begun by United States Department of Agriculture, Agricultural Research Service (USDA-ARS) scientists in 1959 and the Commonwealth Institute of Biological Control (subsequently the International Institute of Biological Control, and now CAB Bioscience) in 1961 (Zwölfer 1969). During the 1970s and 1980s, additional survey work and screening studies on potential *C. solstitialis* bioagents were conducted by USDA-ARS and University

of California at Berkeley entomologists. As a result of these investigations, a complex of capitulum-infesting arthropods was eventually imported into and released in the western United States (Turner and Fornasari 1995, Turner et al. 1995). Six USDA, Animal Plant Health Inspection Service, Plant Protection Quarantine (USDA-APHIS-PPQ) approved insects have been intentionally released in Arizona, California, Idaho, Oregon, and Washington, and another one was unknowingly released (Balciunas and Villegas 1999) and has now established in California, Idaho, Oregon, and Washington.

A capitulum gall-forming fly from Italy, *Urophora jaculata* Rondani (Diptera: Tephritidae), was the first bioagent released in the United States for *C. solstitialis* control (Zwölfer 1969, Sobhian and Zwölfer 1985). Researchers involved with the importations actually believed the insect was *Urophora sirunaseva* (Hering), a closely related species. Introductions of *U. jaculata* against California yellow starthistle biotypes made in 1969, 1970, 1976, and 1977 failed to establish (White and Clement 1987, Turner et al. 1995).

In 1984, the “true” *U. sirunaseva* was first released in the United States in California using flies from Greece, and in Idaho with flies from Turkey (Turner et al. 1995). These and subsequent releases of the insect made in 1985 in California, Idaho, Oregon, and Washington resulted in successful establishment (Turner et al. 1994). This fly completes two generations a year. First generation adults appear during May and early June; second generation individuals emerge in early July. Females deposit one or more eggs atop the partially exposed florets of buds. Over 160 eggs may be laid by an individual female during her lifetime (Sobhian 1993). Larvae burrow through the florets and, upon reaching the receptacle, feed upon immature achenes. In response to *U. sirunaseva* presence, the plant produces lignified tissue which soon envelops the larvae to form distinctive unilocular galls open at the distal end (Zwölfer 1965, Turner et al. 1994). Usually, from one to three galls, but occasionally up to 12 (Turner et al. 1994), form in a capitulum. Infested capitula yield only about 50% of their potential seed crop (Sobhian and Zwölfer 1985). Second generation larvae overwinter inside thick-walled galls within capitula that remain intact or within galls that fall to the soil surface once the capitula disintegrate due to weather-related events. Pupation occurs during late spring (Zwölfer 1965).

A third yellow starthistle herbivore, the weevil *Bangasternus orientalis* (Capiomont) (Coleoptera: Curculionidae), was obtained from Greece and released in the western United States beginning in 1985. Adults congregate on budding plants in early summer and mated females affix individual eggs to scale leaves subtending early-stage capitula. A female is capable of producing over 400 eggs during a three month period (Sobhian and Zwölfer 1985). Upon hatching, the larva enters the leaf mesophyll, mines down the leaf, enters the peduncle, and finally penetrates the receptacle of the bud (Maddox et al. 1986). Several larvae can occupy the capitulum, with each larva being capable of destroying 42-65% of the seeds through its feeding activity (Maddox et al. 1991). Upon the completion of feeding, the larva constructs a chamber from seeds and frass within the capitulum in which it then pupates. Newly formed adults escape from the heads and seek protected overwintering sites. There is but one generation completed annually.

A fourth insect, the tephritid *Chaetorellia australis* Hering, was obtained from Greece and released against *C. solstitialis* in California in 1988 and in Idaho, Oregon, and Washington in 1989. This phytophage can complete three generations annually. First generation adults oviposit into the capitula of bachelor's button (*Centaurea cyanus* L.); second and third generation adults attack both *C. cyanus* and *C. solstitialis*. Female flies deposit their eggs beneath the bracts of late-stage closed capitula, with only a single egg being laid per flower head. A female may eventually produce over 240 eggs during a 60+ day oviposition period



(Sobhian and Pittara 1988). The larva tunnels through the bracts and florets to reach the receptacle where it feeds upon the immature achenes, eventually destroying the majority of them (Sobhian and Pittara 1988). Interestingly, recent studies have shown that *C. cyanus* appears to be the preferred host of *C. australis* (G. L. P., unpublished data). Over a two-year period, infestation of *C. cyanus* heads ranged between 55.2 and 71.2% whereas *C. solstitialis* infestation rates were only 5.6 to 6.6%. Pupation occurs within the capitulum in a cocoon prepared from severed pappus hairs and achene fragments. Third generation larvae overwinter within the heads, pupating during the early spring to yield the first generation adults.

A fifth insect, another European *Chaetorellia* species, *C. succinea* (Costa), has been discovered attacking yellow starthistle populations in northern California and the Pacific Northwest. Adults of this species very closely resemble those of *C. australis*. The fly's biology is also very similar to that of *C. australis* except that *C. succinea* does not require *C. cyanus* as an early season host. It was subsequently discovered from voucher records that a 1991 shipment of *Chaetorellia* from Greece containing both species was mistakenly cleared through the USDA-ARS Albany, California quarantine facility and shipped to Oregon for field release (Balciunas and Villegas 1999). *Chaetorellia succinea* readily established and has rapidly spread to other *C. solstitialis* populations and become a significant capitulum-infesting bioagent. Recent studies have revealed that over 50% of the yellow starthistle capitula collected from various sites in California and throughout the Pacific Northwest are attacked by this fly (J. Balciunas, personal communication). A single larva will destroy most of the seeds in a capitulum (Balciunas and Villegas 1999). Because *C. succinea* was not an intentionally introduced bioagent, its host plant range had not been completely determined. There was some concern that due to its close taxonomic relationship with still another *Chaetorellia* species, *C. carthami* Stackelberg, a known pest of safflower (*Carthamus tinctorius* L.), *C. succinea* also may utilize this crop plant as a host. Based on studies undertaken in California over the last several years, this does not appear to be the case as *C. succinea* could only be reared from yellow starthistle (J. Balciunas, personal communication).

A sixth insect, *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae), another beetle originally collected in Greece, was first liberated in the western United States in 1990. It has since established itself in Arizona, California, Idaho, Oregon, and Washington. *Eustenopus villosus* adults begin to appear during late June and early July. Adult males and females injure yellow starthistle by feeding upon the contents of unopened buds. This activity results in a significant amount (75+%) of bud mortality (Fornasari and Sobhian 1993). Adults also feed on the stems near the capitula, causing the stems to wilt above the feeding point (Turner et al. 1995). Mated females select larger unopened capitula in which to deposit their eggs. A hole is chewed through the capitulum wall and into the floret/achene region. The female deposits a single egg within the cavity and then seals the entry hole with a frass plug. Upon hatching, the larva consumes 70-100% of the developing achenes, the amount varying with capitulum size (Fornasari and Sobhian 1993). In a laboratory study conducted in Europe by Fornasari et al. (1991), the overall reduction in achenes per plant approached 98%. In the United States, 100% seed destruction has been observed within infested capitula (G. L. P., unpublished data). Within the damaged capitulum, the last instar larva constructs a chamber in which pupation occurs. The adult escapes from the chamber in late summer and seeks an overwintering site among soil detritus (Fornasari and Sobhian 1993). *Eustenopus* is a univoltine species.

*Eustenopus villosus* adults appear to exhibit limited powers of dispersal so human-assisted distribution appears to be essential if areawide coverage of yellow starthistle infestations is desired. Capitulum destruction by this species has substantially impacted *C. solstitialis* population development at sites in California, Idaho, Oregon, and Washington since its initial release in these states. The weevil is an excellent bioagent but it may negatively impact population establishment and development of several of the mid- to late season capitulum-attacking phytophages also utilized against the weed.

A seventh insect, *Larinus curtus* Hochhut, another univoltine, achene-consuming curculionid, was released in the western United States in 1992. Establishment has been achieved but populations have not yet reached densities that permit beetles to be collected for widespread redistribution. Weevil adults are active during late June and July and feed on yellow starthistle florets and pollen. Eggs are laid between the florets of partially opened capitula. Normally, only a single egg is deposited per capitulum. The larva feeds on the developing achenes, receptacle tissue, and pappus hairs over a four wk period. Seed destruction levels may exceed 96% (Sobhian and Fornasari 1994). Pupation occurs within the damaged capitula. Adults emerge from the capitula during late summer and early fall and seek protected overwintering sites within the vicinity of the weed infestation (Sobhian and Zwölfer 1985). The weevil is a good flyer like *B. orientalis* and may disperse itself widely (Sobhian and Fornasari 1994).

The six insects that have become established appear to have excellent potential to greatly diminish yellow starthistle seed production, especially *C. succinea* and *E. villosus*. Collectively, the oviposition periods of these bioagents bracket the entire period of capitulum development in the weed: from egg deposition on very early, closed capitulum buds by *B. orientalis*; through oviposition on intermediate to late, closed capitula by *C. australis*, *C. succinea*, *E. villosus*, and *U. sirunaseva*; to egg laying in flowering capitula by *L. curtus* (Turner et al. 1995).

Efforts are being continued by USDA-ARS entomologists to identify, study, and clear additional natural enemies that damage the rosette stage of yellow starthistle (J. Balciunas, personal communication). The weed appears to be highly susceptible to mortality factors at this stage in its life cycle. Arthropods attacking the rosette could increase plant mortality or even substantially reduce the number of capitula available for attack by the already introduced assemblage of capitulum phytophages. A survey for *C. solstitialis* rosette phytophages, carried out during 1997 in the Republic of Georgia (part of the former USSR), Greece, and Turkey, identified several possible candidate biocontrol organisms (J. Balciunas, personal communication). The most commonly encountered insects were weevils in the genus *Ceratapion* (Coleoptera: Apionidae), with *C. basicorne* (Illiger) appearing to be the dominant species of those collected. The biology of this beetle was described by Zwölfer (1965) and Clement et al. (1989). Adults of this univoltine apionid appear during March and April. Eggs are laid on the undersides of rosette leaves and beneath the epidermis of leaf midribs near the root crown. Upon hatching, larvae feed upon midrib, root crown, and stem tissues (Clement et al. 1989). The damage inflicted by *C. basicorne* is dependent upon plant vigor and attack intensity, with heavily infested rosettes often being destroyed (Zwölfer 1965). Pupation occurs within damaged root crowns or stems. Host specificity testing is underway to determine the range of plants accepted by the weevil and other *Ceratapion* spp.

Another organism that shows potential for damaging yellow starthistle is the autoecious rust fungus *Puccinia jaceae* Otth var. *solstitialis* (Uredinales: Pucciniaceae). Infections by this pathogen impact plant vigor and diminish root reserves. If both *P. jaceae* var. *solstitialis*

and *C. basicorne* are judged to be safe for use in North America, permission to import and release them will be sought from USDA-APHIS-PPQ.

The use of biological control agents will not quickly reduce *C. solstitialis* infestations, nor totally prevent further spread of the weed. Nevertheless, biological control organisms most definitely should be included as part of a larger integrated vegetation management effort (Piper 1992). An effective yellow starthistle suppression program requires planning, use of appropriate management methods, regular monitoring/evaluation, and persistence. The development of a specific management plan is dependent upon plant invasive status (new or established) and infestation severity, the nature of the resource to be protected, economic, labor, and other constraints. Emphasis should be placed on the use of those practices that interrupt yellow starthistle seed dispersal and longevity, and that minimize habitat perturbations contributing to further yellow starthistle or other weed species reoccupancy of the site being managed.

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## **Squarrose knapweed**

### **From Challenge to Opportunity: Squarrose Knapweed Demonstration Weed Management Area**

#### **Pat Fosse**

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One of our highest priorities as land managers is to maintain or improve the health of the land. Part of our mission is to preserve options for future generations. We generally agree that, to accomplish this, we must use the "best science". When we hear the word science, most of us think of biological science. We have been trained in biological science or we know where to find the answers to many of our questions or to propose new research to unanswered questions. However, when managing biological organisms, such as noxious weeds, that have no boundaries, we must also use the best SOCIAL science in order to be successful in achieving our goals. Cooperative Weed Management Areas (CWMA) offer us the opportunity to use the best social science as well as the best biological science. This presentation will address a brief history, the learning experiences, and the successes of the Squarrose Knapweed Demonstration Weed Management Area in west central Utah.

Squarrose knapweed (*Centaurea virgata* lam. var. *squarrosa* gegl.) is an invasive perennial noxious weed that was introduced into the U.S. from the eastern Mediterranean region. It is a long-lived plant, that is extremely aggressive and competitive. The deciduous seed heads and re-curved bracts on the seed head allow for extremely efficient seed dispersal. The deep taproot of squarrose knapweed allows it to effectively compete with desirable perennial vegetation for soil resources.

During the fall of 1995, four areas in western states were selected as Demonstration Weed Management Areas (DWMAs) by the Bureau of Land Management. "DWMAs are intended to highlight what can be accomplished through cooperative efforts and partnerships and will be used to document successes and failures in order to provide guidance in the development of other weed management areas." The Squarrose Knapweed Management Area was selected as one of the DWMAs. This DWMA includes portions of four counties including Juab, Tooele, Utah and Millard. It was determined through a GIS inventory that at the time this area was designated as a DWMA, there was between 120,000 and 150,000 acres infested with squarrose knapweed. Other noxious weeds including dyer's woad, Scotch thistle, purple loosestrife, spotted knapweed, Russian knapweed and low whitetop also occur within the DWMA. These noxious weeds occur mostly as small isolated patches or individual plants. A Management Plan was developed by a planning and implementation team composed of twelve individuals from different agencies and groups, including representatives from each of the four counties. Common goals and objectives were developed by the planning team. An integrated weed management plan was developed by the planning team, to achieve our common goals.

There are currently 27 partners working together to manage weeds within the Squarrose Knapweed DWMA. The partners, led by the planning and implementation team, have worked together to implement the plan over the past five years. Each year an Annual Operating Plan is developed by the Planning Team. In the Annual Operating Plan, priorities are established, assignments are given, and goals and objectives are re-visited to determine if

anything has been learned to warrant changing our initial plans and to determine if we are still on track to meet our goals.

Our long-term goals are to: (1) educate the general public and internal personnel about the impacts caused by non-native invasive plants, (2) prevent the spread of squarrose knapweed and other noxious and invading weeds, and (3) through integrated weed management practices reduce the infestation of squarrose knapweed, both population and acreage, to a level where biological control, along with proper management practices, will keep the weed in check within the ecosystem.

Through monitoring and research, we have learned a great deal about which methods and actions most effectively and efficiently help us attain our goals and objectives. We have also demonstrated, through results on the ground, how cooperation and partnerships benefit all partners and agencies involved, and how the synergy or momentum created through successful partnerships or teams allow more innovation, creativity, effectiveness, efficiency and flexibility in a project.

We have learned many valuable lessons over the past five years and we have also accomplished an enormous amount of work on the ground. These experiences and accomplishments will be shared in this presentation.

We can be successful in weed management, therefore maintaining or improving the health of the land and preserving options for future generations. However, the ONLY way to succeed in the management of a biological problem, such as noxious weeds, that have no boundaries, is to remove the boundaries in our minds and work together to establish goals, set priorities, develop and implement plans, and achieve results on the ground. Using the best science is critical to success... the best biological science AND the best social science.

Key words: Integrated weed management, education, prevention, *Centaurea virgata*,  
*Centaurea squarrosa*

## **Establishment of Biological Control Agents on Squarrose Knapweed in California**

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### **Abstract**

Two insect species, the weevils, *Bangasternus fausti* (Reitter) (Coleoptera: Curculionidae), and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) were field released on squarrose knapweed, in Lassen County, California during 1998. Both insects are considered established based on appropriate plant damage as well as presence of adults weevils in the field three, 15, and 27 months after field release. Adult weevils also emerged in the laboratory from field samples collected in 1998, 1999, and 2000 confirming completion of three generations on squarrose knapweed at this site. During the three years, populations of *L. minutus* increased dramatically near the release site, infesting over 96% of the seedheads. *Larinus minutus* is also spreading slowly from the release site as adults were found 2 kilometers away in 2000. The weevil, *B. fausti*, established at much lower rate than did *L. minutus*. Both weevils destroy all the seed within attacked heads.

Key words: Biological control, squarrose knapweed, *Centaurea squarrosa*

### **Background**

Squarrose knapweed, *Centaurea squarrosa* Willd (Asteraceae), is one of the few knapweeds with large established populations in California. Spotted and diffuse knapweeds are aggressively treated with chemicals or mechanically removed as soon as they are found. Unfortunately, squarrose knapweed has established extensively in the northeastern corner of California as well as a few isolated sites elsewhere in the state. Although a classical biological control program has never been initiated on squarrose knapweed, it is closely related to both diffuse and spotted knapweed, which have been targets of classical programs. Some, but not all, of the biological control insects approved for release on spotted or diffuse knapweed were tested on squarrose knapweed during the pre-release host-testing phase. We have initiated efforts to field test available knapweed biological control insects on established populations of squarrose knapweed and determine the potential for impacting the larger infestations in California.

### **Methods**

The weevils, *Bangasternus fausti* (Reitter) (Coleoptera: Curculionidae), and *Larinus minutus* Gyllenhal (Coleoptera: Curculionidae) were collected from established field populations in Oregon then released in California during July 1998. The releases occurred in a large infestation of squarrose knapweed near Pittville, in northeastern California. An estimated 2000 *L. minutus* were point released in one portion of the infestation, while 300 *B. fausti* were released approximately 50 meters south of the *L. minutus* release with near continuous knapweeds between. Less than 20 adult 'UV knapweed seed head fly', *Urophora quadrifasciata* Meigen (Diptera: Tephritidae), were released with the *L. minutus*; however, it had previously been detected in the area, presumably from self-migration.

The first field evaluation occurred on October 8, 1998. We found adults of both *B. fausti* and *L. minutus* in the field, as well as feeding damage within seedheads of squarrose knapweed consistent with their attack. Field samples were collected from both sites and



evaluated in the laboratory. One year later, on September 8, 1999, adults of both species were again noticed at the site and within the collected field samples. Samples for both years, consisting of ten plants, were collected along a transect established across the release site. All seedheads were removed from the ten plants, bulked, and subsamples (>300 heads/site) were selected for evaluation. The third collection occurred on September 15, 2000, and all seedheads on all ten plants per site were dissected. The results of the dissections are shown in Table 1.

Two additional collections were made during 2000 in order to investigate the natural spread of the biological control insects. One collection was made 800 m west of the release sites but still within the continuous infestation. A second collection was made 2 km east of the site with only widely scattered plants between. The results of the dissections are shown in Table 1.

Seed destruction caused by the biological control insects was evaluated in 2000 by enclosing 50 post pollination seedheads at each site in small cotton bags tied around the stem. Seedheads were allowed to remain attached to the plant for four weeks to allow maturation of seeds and insects then collected and processed in the laboratory. An additional measure on seed production was obtained by counting seeds in noninfested seedheads collected in September 2000.

### **Results and Discussion**

Both of the weevil species, as well as *Urophora quadrifasciata*, seem well established at this site. All three species are quite capable of maintaining viable populations on squarrose knapweed, although relatively low numbers of seedheads are attacked by *U. quadrifasciata* (Table 1). Approximately 20 adult *Urophora quadrifasciata* were released at the same time as the weevils but the gall fly appears to have migrated to the site prior to the release. The numbers that we detect suggest that it will not likely become a major factor in controlling the weed. The gall fly may, in fact, eventually be eliminated as the weevils spread through the site consuming both seeds and *U. quadrifasciata* larvae.

The weevil, *B. fausti*, has been shown to establish low populations on squarrose knapweed in Utah and at a site near Hawkinsville, California. The population levels reported here, although relatively low, are clearly increasing and are actually quite encouraging for a weed biological control agent during the first three years after release. Adult weevils did emerge from the samples collected in 1998, 1999 and 2000, confirming that *B. fausti* has completed three generations on squarrose knapweed at this site and is likely established.

Adult weevils of *L. minutus* also emerged all three years, confirming that it also established well at this site. Attack rates were high the first year and dramatically increased around the actual release site. Over 90% of the seedheads were attacked by *L. minutus* near the release site, with most of the attacked seedheads being completely destroyed. Two intact seeds were found in one of the 1001 heads attacked by *L. minutus* at the *Larinus minutus* site, but the larvae had died at an early age. *Larinus minutus* is also spreading out from the release site as adults were found at least 50 meters away after one year and 800 meters during 2000.

Uninfested squarrose knapweed produces very few seed per seedhead, usually 1-3 and very rarely 5 or 6. Flowerheads in our studies produced an average of 6.88 fertile flowers and 1.44 seeds per head (Table 2). Enclosing seedheads in cotton bags just after flowering ensured that all seed are captured and counted, and was hoped to provide an accurate evaluation of seed destruction. However, the high attack rate by the weevils meant that of the 88 bagged heads only three were uninfested and produced seed (2, 1, and 1). Seedheads of *C. squarrosa* are deciduous, easily breaking off and attaching to animals or people for

spread. Unlike some species of knapweed, the seedhead remains closed at maturity containing most if not all seeds. Thus as a matter of practicality, unbagged seedheads are a more efficient experimental unit.

Based on these preliminary findings, we feel that the two weevils are well suited to *C squarrosa* and may become one of the most dramatic successes for knapweed biological control. Since these weevils do not spread rapidly, efforts will be continued to distribute these weevils to other squarrose knapweed sites in the state. Additional years of study will also be made to follow knapweed densities.

Table 1. End of season status of biological control on squarrose knapweed near Pittville California. Weevils were released early summer 1998. The gall fly immigrated some time prior to 1998. Number indicates the percentage of seedheads attacked.

| Site and species           | 1998  | 1999  | 2000  |
|----------------------------|-------|-------|-------|
| <i>L. minutus</i> site     |       |       |       |
| <i>Larinus minutus</i>     | 16.3% | 86.5% | 91.3% |
| <i>Bangasternus fausti</i> | 0.6%  | 0%    | 4.1%  |
| <i>U. quadrifasciata</i>   | 4.6%  | 2.4%  | 0.6%  |
| Total infested             | 21.6% | 88.5% | 96.0% |
| <i>B. fausti</i> site      |       |       |       |
| <i>Larinus minutus</i>     | 1.0%  | 6.9%  | 79.3% |
| <i>Bangasternus fausti</i> | 0.3%  | 1.8%  | 9.6%  |
| <i>U. quadrifasciata</i>   | 1.3%  | 0.9%  | 0.2%  |
| Total infested             | 2.6%  | 9.7%  | 89.0% |
| 2 km west                  |       |       |       |
| <i>Larinus minutus</i>     |       |       | 0.8%  |
| <i>Bangasternus fausti</i> |       |       | 0%    |
| <i>U. quadrifasciata</i>   |       |       | 0.5%  |
| Total infested             |       |       | 1.4%  |
| 50 m east                  |       |       |       |
| <i>Larinus minutus</i>     |       |       | 36.8% |
| <i>Bangasternus fausti</i> |       |       | 0.6%  |
| <i>U. quadrifasciata</i>   |       |       | 0%    |
| Total infested             |       |       | 37.2% |

Table 2. Flower and seed production by squarrose knapweed and the impact of seedhead insects

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|  |              |
|--|--------------|
| Unbagged samples                               |              |
| Average number of fertile flowers per seedhead | 6.88 (n=60)  |
| Seeds per uninfested seedhead                  | 1.44 (n=472) |
| Bagged samples                                 |              |
| Seeds per uninfested seedhead                  | 0.8 (n = 3)  |
| Seeds per infested seedhead                    | 0 (n= 88)    |

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## **ABSTRACTS**

### **General Session**

#### **Ecological Principles for Managing Knapweed**

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Knapweed management must move from temporary prescriptive control toward sustainable management based upon ecological concepts and principles. Site-specific prescriptive management often fails because prescriptions were developed under circumstances different from those of the management area. In addition, prescriptions are aimed at treating the symptoms, weeds. Sustainable knapweed management requires employing strategies aimed at manipulating the mechanisms and processes directing plant community dynamics. Less than 10% of our land is dominated by invasive plants, such as knapweed. Although preventing weed movement is commonly mentioned as an important part of weed management, few programs successfully implement effective prevention program. For example, most county programs keep knapweeds from spreading along roadways, but they continue to rapidly move along waterways. Another important consideration is early detection, which at this point is poorly organized and lacks a systematic approach that maximizes the potential for detection. On large-scale infestations, weed management must focus on developing ecologically healthy, weed-resistant plant communities that meet other land use objectives. Successional knapweed management attempts to understand the general causes of plant community change. They are site availability, species availability and species performance. Knowledge of these three causes and their modifying factors provides a basis for ecologically based, integrated knapweed management. Understanding plant demographic data provides ecological information essential to understanding the cause and solutions for weed management by identifying key mechanisms and processes directing plant community dynamics, and allowing the prediction of plant community response to management. This information is central to making wise decisions about knapweed management. Finally, R\* theory (Tilman, 1985, *Am. Nat.* 125: 827-852) offers the potential for useful principles based on how the competitive relationships among species are changed by various management practices. Ultimately, the goal is to use technology to manipulate the mechanisms directing succession toward a desired plant community based on ecological principles.

## Biological Control of Russian Knapweed: State of the Art

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First investigations on the prospects of classical biological control of Russian knapweed started in the 1970s and led to the release of a gall-forming plant parasitic nematode, *Subanguina picridis*. Although this nematode can have considerable impact on Russian knapweed under specific conditions, it did not prove to be an effective agent under field conditions. Starting in 1996, new efforts have been undertaken to find and study biological control candidates originating from various parts of the native range in Asia. An overview will be given on the results of the first four years of the “new” biological control program, addressing the following aspects: a) what type of herbivory is likely to affect Russian knapweed population dynamics, b) what herbivores are associated with Russian knapweed in its native range, and c) what is known about the biology and host-specificity of the first three shoot- or root-attacking herbivores under investigation (i.e. the gall-wasp *Aulacidea acroptilonica* (Cynipidae), the fly *Napomyza* sp. near *lateralis* (Agromyzidae), and the moth *Cochylimorpha nomadana* (Cochylidae)).

Key words: *Acroptilon repens*, *Subanguina picridis*, *Aulacidea acroptilonica*, *Napomyza* sp. near *lateralis*, *Cochylimorpha nomadana*, biological control, insects

## Integrated Approaches for the Management of Yellow Starthistle

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A number of control options are available for the management of yellow starthistle, including grazing, mowing, clover or perennial grass reseeding, burning, chemical, and biological control. Recent studies have emphasized the development of integrated systems for the long-term sustainable management of yellow starthistle. Such systems include various combinations of a number of these newly developed techniques. The objective of using an integrated approach is to provide ranchers and land managers with economical and sustainable management programs that maximize forage quality and quantity or preserve ecosystem integrity, while also reducing the susceptibility of their lands to re-invasion or invasion by other noxious weeds. One such study combines herbicides, biological control, and competitive perennial grass reseeding. The goal of this revegetation project is to develop sustainable high quality range conditions and improved wildlife habitat capable of providing

long-term starthistle control without the need for continued herbicide treatments. Based on the findings of this study, yellow starthistle seedbanks might allow infestations to readily overcome one and possibly two years of clopyralid treatment. However, on severely degraded rangeland an integrated combination of clopyralid treatment and wheatgrass seeding can be very effective in suppressing yellow starthistle seed production and may provide a more effective long-term solution than applying clopyralid alone. This strategy is also compatible with the survival of yellow starthistle biocontrol agents. It is hoped that the insects will maintain low starthistle seed production, further slowing the re-infestation rate. Other integrated studies are investigating the effectiveness of integrating summer prescribed burning and clopyralid treatment into yellow starthistle management programs.

Key words: *Centaurea solstitialis*, IPM, rangeland

## **Comprehensive Interactive Plant Keys for the Northwest**

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Interactive keys for the computer are demonstrated for all the vascular plants of Washington, Oregon, Idaho, southern BC, Montana, Wyoming, Utah and Colorado. The keys include 6500 species, whether native or naturalized, weedy or not. The keys allow the user to identify easily any plant in a fraction of the time normally required. A person may select which characteristics of a plant to use in keying it out, and in any order, instead of being limited to the forced choices of a dichotomous key. The user may also ask the system to provide suggestions of what to examine on the specimen, based on the current input and species remaining in the database. Identification is made by the plant's unique combination of characteristics, and with a little practice takes one to two minutes. Confirmation of the identification may then be validated by the references listed in the program, and by color images of the plants.

Key words: interactive plant keys

## **Spotted and Diffuse Knapweed – revegetation and competition**

### **Establishing Desirable Grass in Spotted Knapweed Infestations Using High Seeding Rates**

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Long-term and sustainable management of rangeland degraded by spotted knapweed will require establishment of desirable grasses. Revegetation of weed infested rangeland is risky and development of reliable grass establishment is needed. The overall objective was to improve grass establishment in spotted knapweed infestations using high seeding rates. The specific objective was to compare intermediate wheatgrass establishment at 4 seeding rates, in combination with tillage and/or glyphosate (n-phosphomethyl glyine), in spotted knapweed infested rangeland. We hypothesized that the establishment of intermediate wheatgrass would be greatest at high seeding rates, while spotted knapweed density and biomass would be negatively impacted by intermediate wheatgrass. Glyphosate (1.16 liters a.i./ha; with and without), tillage (200 mm depth; with and without), and 4 seeding rates (0, 500, 2,500, 12,500 m<sup>-2</sup>) of intermediate wheatgrass seeds were factorially arranged in a randomized-complete-block design with 4 blocks at each of 2 sites in Montana. Treatments were applied in the fall of 1995. By the second growing season, intermediate wheatgrass failed to established in plots seeded with 500 seeds m<sup>-2</sup>, the currently recommended seeding rate. Increasing the seeding rate to 2,500 and 12,500 m<sup>-2</sup> increased intermediate wheatgrass tiller density by 80 and 140 plants m<sup>-2</sup>, respectively, at Hamilton and 158 and 710 plants m<sup>-2</sup>, respectively, at Bozeman. At the highest seeding rate, combining tillage with glyphosate increased tiller density over 3 times more than other treatments where intermediate wheatgrass successfully established at Hamilton. However, neither tillage nor glyphosate affected intermediate wheatgrass density at Bozeman by the second growing season. Fifth year results will be presented. Our revegetation study suggests that increasing intermediate wheatgrass seeding rates can facilitate their establishment in spotted knapweed infested rangeland and may enhance our ability to use revegetation as an effective weed management strategy.

Key words: spotted knapweed, intermediate wheatgrass, revegetation, tillage, glyphosate, seedling establishment

### **Developing Single Entry Revegetation of Spotted Knapweed-infested Rangeland**

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Establishing competitive plants is essential for successful management of spotted knapweed infestations where desirable vegetation is absent. Our objective was to determine a herbicide

or herbicide-combination that would maximize grass establishment in spotted knapweed-infested rangeland in a simulated single fall application. On 2 sites in Montana, 8 herbicide treatments at 0.5 kg a.i. ha<sup>-1</sup>, picloram at 0.14 kg a.i. ha<sup>-1</sup>, picloram at 0.28 kg a.i. ha<sup>-1</sup>, clopyralid at 0.21 kg a.i. ha<sup>-1</sup> plus 2,4-D at 1.12 kg a.i. ha<sup>-1</sup>, picloram at 0.14 kg a.i. ha<sup>-1</sup> plus glyphosate at 0.5 kg a.i. ha<sup>-1</sup>, picloram 0.28 kg a.i. ha<sup>-1</sup> plus glyphosate at 0.5 kg a.i. ha<sup>-1</sup>, and clopyralid 0.2 kg a.i. ha<sup>-1</sup> plus 2,4-D at 1.12 kg a.i. ha<sup>-1</sup> plus glyphosate 0.5 kg a.i. ha<sup>-1</sup>] were applied and 3 grass species (Luna pubescent wheatgrass, bluebunch wheatgrass, and Bozoiski Russian wildrye) were seeded in a spilt-plot design with 4 replications in the late-fall of 1994 and 1995. Spotted knapweed and grass density were measured in 1995, 1996, and 1997. Biomass was measured in 1997. Density data were analyzed as a split-split-plot in time. Biomass data were analyzed as a split-plot using analysis of variance. By the end of the study, picloram (0.14 or 0.28 kg a.i. ha<sup>-1</sup>) consistently resulted in the lowest spotted knapweed density and biomass. Initially, glyphosate alone lowered spotted knapweed density and increased grass biomass compared to that of the control. However, glyphosate treated plots had more spotted knapweed and less seeded grass established by the end of the study. 'Luna' pubescent wheatgrass consistently yielded the highest density and biomass of the seeded grasses. We believe a single-entry revegetation program applying picloram in late-fall combined with a fall-dormant seeding will maximize grass establishment in spotted knapweed infested rangeland.

Key words: spotted knapweed, Luna pubescent wheatgrass, bluebunch wheatgrass, Bozoiski Russian wildrye, rehabilitation; weed management; re-seeding; seedling establishment

## **Native Bunchgrass Community Restoration Using Knapweed Herbicides**

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The Sawmill Research Natural Area was established by the US Forest Service in 1987 as the best remaining example of Idaho fescue/bluebunch wheatgrass and rough fescue/ Idaho fescue habitat types on the Bitterroot National Forest (Ravalli County, Montana). This mountain foothill site is used heavily by elk as winter range. In years subsequent to the RNA designation it was noted that spotted knapweed density and acres infested were increasing markedly within the management unit, and native bunchgrasses and forbs were declining in abundance. In 1995 replicated Daubenmire plots were established in several habitat types, and baseline measurements of full community composition were made. The replicated test plots were sprayed with 1 pint/acre Tordon 22K in the fall of 1996. Canopy cover response of all species was determined in 1998 and 1999. Production of habitat type indicator bunchgrasses was greatly increased by spraying and the impact on non-target forbs was minimal. Floristic trajectories for the test plots were calculated by nonmetric multidimensional scaling and compared to the mean composition of the Mueggler and Stewart (1980) habitat type definitions for these bunchgrass communities. The herbicide treatments drove the species composition towards the potential natural community as defined by using the Mueggler and Stewart data sets as quantitative reference standards. This favorable response confirmed improvement in the similarity of sprayed knapweed infested plots to the potential natural communities at other sites as analyzed by ordination techniques



and previously reported by Rice and Toney (1998). The susceptibility of spotted knapweed to low rates of picloram and clopyralid allows the use of these herbicides for restoring native communities on sites with high conservation value.

Key words: ordination, picloram, clopyralid, restoration, bunchgrasses, spotted knapweed, elk

## **Nitrate Uptake of Spotted Knapweed and Two Native Grasses from Pulse Events**

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Spotted knapweed (*Centaurea maculosa*) displaces native semiarid grasses in the foothills and valleys in the Northern Rocky Mountain region, possibly because spotted knapweed competes more effectively for nutrients, especially nitrogen. In semiarid areas, N is often available only during short pulse events associated with soil wetting. Our objective was to determine whether spotted knapweed takes up more N during pulse events than bluebunch wheatgrass *Pseudoroegneria spicata* or western wheatgrass *Pascopyrum smithii*. In a greenhouse, spotted knapweed was grown with each of these native grasses in small pots, and each species was grown in monoculture. After they were established, these five species combinations were conditioned to either 12, 24, or 72 h pulse events of N availability each week for 8 weeks. Then pots were labeled with  $^{15}\text{NO}_3$  for 8 h, and harvested 24 h later. Western wheatgrass plants conditioned to 24 and 72 hour pulse events had greater root and shoot mass than spotted knapweed; conversely, spotted knapweed plants conditioned to 24 and 72 h pulse events had greater root and shoot mass than bluebunch wheatgrass. Across all three pulse durations, western wheatgrass acquired more  $^{15}\text{NO}_3$  than spotted knapweed; conversely, spotted knapweed acquired more  $^{15}\text{NO}_3$  than bluebunch wheatgrass. These results indicate that spotted knapweed's success at displacing bluebunch wheatgrass may be attributed, in part, to its ability to more effectively take up N. They also indicate that reseeding western wheatgrass into spotted knapweed infested areas may have a greater likelihood of success than reseeding bluebunch wheatgrass.

Key words: competition, native grasses, nitrate uptake, *Centaurea maculosa*, *Pseudoroegneria spicata*, *Pascopyrum smithii*

## **Influence of Nutrient Availability on the Interaction between Spotted Knapweed and Native Perennials**

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Manipulating nutrient availability has been suggested as a mechanism for accelerating succession away from plants with characteristics of early and mid-seral species. Early successional plant communities often thrive in sites with high nutrient availability, whereas late successional plant communities are often found on sites with lower nutrient availability. This study tested the ability to direct succession away from spotted knapweed by altering nutrient availability. We hypothesized that depletion of nutrients would switch the competitive advantage from spotted knapweed to bluebunch wheatgrass and nutrient addition would favor spotted knapweed. Background densities of annual rye and bottlebrush squirreltail were used to remove nutrients from the soil. Nutrient addition was accomplished by nitrogen or phosphorus amendments. Data were fit to Watkinson's curvilinear model to determine the competitive relationship between bluebunch wheatgrass and spotted knapweed. Competition coefficients indicated that without nutrient manipulation, spotted knapweed was more competitive than bluebunch wheatgrass. Annual rye additions changed the competitive balance in favor of bluebunch wheatgrass. Addition of nitrogen, phosphorus, or bottlebrush squirreltail did not change the competitive relationship between the two species. This study indicated that succession from spotted knapweed toward native plants might be accelerated by reducing resource availability.

Key words: nutrient availability, bluebunch wheatgrass, annual rye, bottlebrush squirreltail, nitrogen

## **Effects of Season and Frequency of Mowing on Spotted Knapweed and Grasses**

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Spotted knapweed (*Centaurea maculosa* Lam.) is a non-indigenous weed that has invaded millions of hectares of rangeland in the United States. Mowing may be a useful tool for reducing knapweed. Our objective was to study the response of knapweed and grasses to mowing timing and frequency. The response of knapweed to 16 mowing treatments and two clipped biomass treatments repeated annually for three years was studied at two sites. Mowing treatments consisted of combinations of spring, summer and fall mowing. Clipped biomass treatments consisted of a mulch-mown treatment and a treatment in which clipped biomass was collected and removed from plots. Mowing and clipped biomass treatments were arranged in a randomized complete block design with four replications (16 mowing; 2 clipped biomass; 4 replications; 2 sites = 256 plots). In most instances a single fall mowing when knapweed was in the flowering or seed producing stage reduced knapweed cover and adult density as much as any treatment consisting of repeated mowing. Fall mowing

decreased adult density an average of 73 and 41% below that of the unmown control at site 1 and 2, respectively. Mowing treatments reduced seedling density 0 to 99% below that of the unmown control, but the response was inconsistent unless the treatment consisted of frequent mowing. Removal of clipped biomass decreased knapweed seedling density 39% below that of the unmown control at one site in one year. Knapweed cover and adult density were severely decreased by mowing, while grass cover was only decreased by a few mowing treatments at one site in one year. We hypothesize that annually repeated mowing may shift the competitive balance in favor of desired grasses.

Key words: mowing, spotted knapweed, *Centaurea maculosa*

**Mechanisms for the Success of Invaders:  
Diffuse Knapweed Interacts Differently with New Neighbors than with Old Ones**

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The success of some invasive plants is generally attributed to escape from enemies, freeing them to compete fully. This perspective provides the theory for the practice of introducing natural enemies as biological controls to suppress invasive plants. However, the success of some invasives may also be due to novel mechanisms of interaction.

We compared the effects of *Centaurea diffusa* on three bunchgrass species that co-exist with *C. diffusa* in Eurasia to the effects of *C. diffusa* on three bunchgrass species from North America with similar morphologies and sizes, each of which is closely related to one of the Eurasian grass species. *Centaurea diffusa* had stronger negative effects on North American species than it had on Eurasian species. Correspondingly, none of the North American grass species (nor all species analyzed collectively) had a significant competitive effect on the biomass and <sup>32</sup>P uptake of *C. diffusa*, but the Eurasian species *Koeleria laerssenii*, and all Eurasian species analyzed collectively, significantly reduced *C. diffusa* biomass and <sup>32</sup>P uptake.

Activated carbon added to ameliorate chemical effects had contrasting effects on the interactions between *C. diffusa* and grass species from the different continents. When growing with *C. diffusa*, the biomass of two North American species, *Festuca idahoensis* and *Pseudoroegneria spicata*, increased with activated carbon added to the soil and the overall effect of carbon on North American species in competition with *C. diffusa* was positive. In contrast, the biomass of all Eurasian grass species growing with *C. diffusa* was reduced in the presence of activated carbon. These effects are evidence for stronger allelopathic effects of *C. diffusa* on North Americans than on Eurasians and suggest that some invasive plants may use competitive mechanisms not present in the natural communities that they invade to disrupt interactions among long-associated native species.

Key words: *Centaurea diffusa*, *Koeleria laerssenii*, *Festuca idahoensis*, *Pseudoroegneria spicata*, competition, allelopathy, biogeography, phosphorus

## **Restoring Natural Areas with Successful Diffuse Knapweed Control**

**Berta Youtie**

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After 14 years of battling diffuse knapweed I can offer a success story and some hope. I manage Nature Conservancy natural areas in central and northeast Oregon most of which are infested with knapweed species. We use an integrated weed control program consisting of mechanical and chemical methods.

Though initially more labor intensive, we have found that mechanical control is effective for controlling small populations of diffuse knapweed scattered amongst native plants. In the 1980's the Tom McCall Preserve had a small, often dense infestation of diffuse knapweed. Numerous volunteers from the Portland metropolitan area logged about 360 person-hours in 1989 removing thousands of plants from the preserve and adjacent Oregon State Park. Each year rosettes were dug in early May, bolted plants in June, and all remaining plants dug and bagged in July reducing the population 98% by 1994. About 10 person-hours are still required each season to survey and remove a few dozen plants that sprout from old seed buried in the ground.

We apply a combination of chemical and mechanical methods to control larger infestations. On the Boardman Research Natural Area the largest remnant of shrub steppe habitat in the Oregon Columbia Basin, diffuse knapweed is spread over 1500 acres. Staff personnel apply picloram from backpack sprayers in April followed by Americorp crews (~10 people) who walk the area at six foot intervals mechanically removing plants missed by the spray crew. We try to cover the entire infested area twice each season which reduces seed production substantially. Knapweed within the Natural Area is greatly reduced, however, a significant population remains in the buffers due to recruitment from upwind seed sources. Successful weed control must incorporate all surrounding landowners.

Key words: knapweed control

**Spotted and Diffuse Knapweed – biological control and taxonomy**

**How to Select Optimal Sites for Establishment of *Agapeta zoegana* and *Cyphocleonus achates*, Two Root Feeding Insects of Knapweed**

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Selecting sites with certain characteristics can increase the successful establishment of biological control agents. A wide range of site and insect characteristics were evaluated at 125 spotted knapweed sites in Montana, northern Idaho, and eastern Washington to identify important factors in the establishment of *Agapeta zoegana* and *Cyphocleonus achates*. Knapweed infestation type, such as continuous, patchy, or linear, and soil type significantly contributed to the establishment of *A. zoegana*. More insects were recovered in continuous infestations and on sandy clay loam and clay loam soils. Additional factors to consider when selecting sites to release *A. zoegana* are a minimum of 100 insects per site on at least one acre of knapweed with little to no bare soil, minimal site disturbances, and larger knapweed plants. For *C. achates*, significant factors affecting insect establishment were multiple releases of insects over time, knapweed infestation size and type, and elevation. More insects were recovered at sites with a minimum of five continuous acres of spotted knapweed, elevations of 3,000-5,000 feet, and at sites where insects were released over multiple years. Additional factors to consider when selecting sites to release *C. achates* are a minimum of 200 weevils per site, moderate knapweed densities, minimal disturbances, and larger knapweed plants.

Key words: site characteristics, spotted knapweed, *Agapeta zoegana*, *Cyphocleonus achates*

**Direct and Indirect Effects of Well-Behaved Biological Control Agents on Nontarget Species - a Case Study: Spotted Knapweed, Gall Flies, Deer Mice**

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Spotted knapweed (*Centaurea maculosa*) is one of the most widely established invasive exotic plants in Northern Rocky Mountain ecosystems. Spotted knapweed invasion results in virtual monocultures that greatly reduce species richness of native plant communities with largely unknown consequences for native fauna. Although spotted knapweed responds well to herbicide applications, its establishment over vast acreages of grasslands and savannas precludes effective control of this noxious weed by means of herbicide treatment. As a result, spotted knapweed has been heavily targeted for control by means of classical biological control measures and so provides a case study for examining the potential influences of biological control on native ecosystems. Biological control agents have successfully controlled some prominent exotics, but such successes are rare and debate has

ensued regarding the appropriateness of biological control. This debate has focused on the potential for biological control agents to shift herbivory, predation, or pathology to nontarget species, but has largely ignored the potentially complex impacts of well-behaved (i.e., host-specific) biological control agents. We present data suggesting that complex nontarget effects can result from well-behaved biological control agents. Small mammal studies in spotted knapweed invaded grasslands indicate that deer mice (*Peromyscus maniculatus*) seasonally alter their diets and habitat selection to exploit the larvae of gall flies (*Urophora* spp.), exotic insects released to control spotted knapweed. Changes in deer mouse ecology may affect small mammal communities, small mammal predators, and the roles small mammals play in the ecology of exotic plant invasions. Our results indicate that even well-behaved biological control agents may significantly impact native communities through complex indirect effects. The potential for such indirect effects should be considered in the process of choosing biological control insects for management of exotic species.

Key words: biological control, Spotted knapweed, *Centaurea maculosa*, Deer mice, *Peromyscus maniculatus*, *Urophora* spp., indirect effects

### **Knapweed, Gall Flies, and Mice: Unexpected Interactions**

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Two species of seedhead gallflies (*Urophora affinis* and *U. quadrifasciata*) were introduced for knapweed biocontrol almost 30 years ago. Although both species have become widespread and abundant, there has been no appreciable reduction in spotted knapweed density or rate of spread. Deermice (*Peromyscus maniculatus*) can consume 50-75% of overwintering gallfly larvae. Does this extensive predation reduce the gallflies effectiveness as a biocontrol? Alternatively, did the gallflies fail because even at peak densities they cannot reduce seed production below some necessary threshold? If so, could any seed predator ever be an effective biocontrol for knapweed? I address these questions through exclosure experiments, where I monitor the response of gallflies and knapweed to rodent exclusion. I incorporate results from these experiments in a demographic model of spotted knapweed, to translate the impacts on plant performance to changes in population dynamics.

Key words: *Urophora*, *Peromyscus*, community ecology, seed production, biological control

## **Are Biological Controls Effective Against Knapweed?: Neighboring Plant Determines Compensatory Response of Spotted Knapweed**

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Grazing and biocontrol insects are currently used to reduce the spread of invasive *Centaurea* species. These methods are based on the assumption that herbivory will reduce the competitive effects of *Centaurea* on native plants. In a greenhouse experiment, we studied the effects of herbivory on the interactions between *C. maculosa*, and two native grasses, *Festuca idahoensis* and *Festuca scabrella*. *Centaurea maculosa* was grown alone or in pairs with *C. maculosa*, *F. idahoensis*, or *F. scabrella*. We applied *Trichoplusia ni* to one *C. maculosa* in each treatment and left others without herbivores as controls. Herbivory had a negative effect on the total biomass of the target *C. maculosa* in the absence of competition, and the target *C. maculosa* equally compensated for herbivory in the presence of a neighbor. Herbivory did not reduce competition on neighboring plants.

We also conducted an experiment to determine if *C. maculosa* responds to herbivory differently when planted with the American grass, *F. idahoensis*, versus when planted with the European grass, *F. ovina*. We planted *C. maculosa* alone or with a neighboring *C. maculosa*, *F. idahoensis* or *F. ovina*. To obtain consistent, quantifiable levels of herbivory, we simulated herbivory by clipping the target *C. maculosa* with scissors. The target *C. maculosa*'s growth response to herbivory was strongest when planted with another *C. maculosa*. *Centaurea maculosa* equally compensated when planted with *F. idahoensis*, but not with *F. ovina*, suggesting that interactions between *C. maculosa* and European neighbors differ than interactions between *C. maculosa* and American neighbors. These results contradict two assumptions of biological control: 1) the role of herbivory in *C. maculosa*'s native range is the same as in its invasive range, and 2) herbivory will decrease the competitive ability *C. maculosa*. This could have important implications for the use of herbivory as a biological control.

Key words: *Centaurea maculosa*, *Festuca*, biological control, compensatory growth

## **Effects of the Interaction of the Biocontrol Agent, *Agapeta zoegana* L., and Grass Competition on Spotted Knapweed**

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*Agapeta zoegana* L. is a Eurasian root-mining moth introduced for biological control of spotted knapweed, *Centaurea maculosa* Lamarck, in North America. A study was conducted during 1992 through 1994 to make a preliminary assessment of the combined effects of the moth and grass competition on spotted knapweed plant structure and density at two nearby

sites in western Montana. Knapweed plants at the *A. zoegana* release site had less above-ground biomass (43%), fewer stems per plant (29%), fewer capitula per plant (43%), and were shorter (18%) than knapweed plants at the check site (Story et al. 2000). A comparison of infested versus uninfested knapweed plants throughout both sites showed that infested plants had more stems (15%), more capitula (40%), more above-ground biomass (112%), were taller (7%), had thicker roots (92%) and were older (22%) than uninfested plants, suggesting the moth preferentially attacks older, larger knapweed plants. Numbers of *A. zoegana* larvae per root were positively correlated with root diameter. Incidence of attack by *A. zoegana* was significantly greater in bolted knapweed plants than in rosettes, but larvae showed no preference for bolted plants over rosettes when root diameters were similar. *Agapeta zoegana* had no effect on knapweed rosette density, but appeared to reduce the number of bolted knapweed plants in plots with low grass density (10% grass cover) by 39 percent, and increase the density of knapweed seedlings in the spring by 65 percent. Knapweed plants in plots with high grass density (50% grass cover) were shorter, had less above-ground biomass, and had fewer capitula, compared to plots with low grass density. The impact of *A. zoegana* on spotted knapweed was not enhanced by grass competition.

References:

Story, J.M., W.R. Good, L.J. White, and L. Smith. 2000. Effects of the interaction of the biocontrol agent, *Agapeta zoegana* L. (Lepidoptera: Cochylidae), and grass competition on spotted knapweed. *Biological Control* 17:182-190.

Key words: *Agapeta zoegana*, biological control, weed, rangeland, grass competition, spotted knapweed, *Centaurea maculosa*

**How to Monitor Spotted Knapweed Biological Control Root Feeding Insects: *Agapeta zoegana* and *Cyphocleonus achates***

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Effective monitoring methods for biological control agents of weeds are essential to evaluate establishment, dispersal, and impact of insects on weed populations. Several monitoring methods were evaluated for both adults and larvae of *Agapeta zoegana* and *Cyphocleonus achates*. For *A. zoegana*, pheromone traps, visual transects, sweep netting for adults, and root excavation for larvae were evaluated at sites across Montana, eastern Washington, and northern Idaho. Pheromone traps recovered the most moths at sites evaluated. For *C. achates*, visual searching and sweeping for adults, and root excavation for larvae were evaluated. Larval sampling recovered the most weevils across sites evaluated. Pheromone trapping for *A. zoegana* and larval sampling for *C. achates* are effective monitoring tools in biological control programs.

Key words: monitoring methods, *Agapeta zoegana*, *Cyphocleonus achates*



## **Biology and Biological Control Agents of the Knapweeds, a Reference for Biological Control Programs**

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This book, the second in a series, will summarize the current state of the art detection and evaluation strategies for biological control agents currently available and approved for redistribution on weedy knapweed species. The book is being prepared specifically for weed managers who may not be familiar with biological control, insect biology, or how biological control may compliment existing weed management strategies. It will include sections on biology of the weedy knapweed species, biology of root feeding biological control agents, biology of seed head feeding biological control agents, and how the biology of plants and agents affects such processes as monitoring for agent presence and evaluating the impact of biological control agents on the target weed population. Information will be presented in a variety of formats to assist managers with basic tasks such as determining agent presence or absence, choosing appropriate release sites, and conducting vegetation monitoring. The University of Idaho is developing this book in cooperation with the USDA Forest Service and a host of knapweed researchers.

Key words: biological control, monitoring, plant biology, insect biology, biological control program management

## **Molecular Markers for *Centaurea* Population Genetics**

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To manage invasive plant populations effectively, we must understand the fundamental principles governing their spread. By measuring the extent of genetic diversity within and between native and introduced populations of invasive weeds, we can gain a better understanding of how genetic traits influence the invasion of exotic plants into our rangelands and wilderness areas. Furthermore, molecular markers may be used to pinpoint the origins of invasive weeds, facilitating the search for effective biological control agents. I am developing markers for exploring the genetics of diffuse and spotted knapweeds (*Centaurea diffusa* and *C. maculosa*). I will report on the utility of Inter Simple Sequence Repeat (ISSR) markers, microsatellite markers, and restriction fragment length

polymorphisms in amplified segments of the chloroplast genome (PCR-RFLP) for population genetics of these noxious weeds.

Key words: *Centaurea diffusa*, *Centaurea maculosa* genetics, introduction, weed origins, biological control

### **An Overlooked Knapweed Hybrid in North America: *Centaurea* × *psammogena* GÁYER (Diffuse Knapweed × Spotted Knapweed)**

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During recent studies on spotted knapweed (*Centaurea stoebe* L.) in Europe it became quite obvious that fertile hybrids with the closely related diffuse knapweed (*Centaurea diffusa* LAM.) are far more frequent than expected. Both parents are noxious weeds in North America, and although there are suspicions that the hybrid occurs there, there are no definitive reports in the literature. A review of American literature on invasive knapweed species provided evidence that this hybrid occurs in North America: in two cases figures labelled diffuse knapweed clearly showed characters of the hybrid. Studies in several herbaria (including MO and MICH) revealed a number of specimens of *C. × psammogena* from seven different states of the USA.

Two facts may be of importance for biological control of spotted knapweed, diffuse knapweed and their hybrid: 1) Introgression of diffuse knapweed into spotted knapweed was proven by molecular data. This may result in a change of ecological characters and special adaptations. 2) While all American plants of spotted knapweed are reported to be tetraploid ( $2n = 36$ ), only diploid plants ( $2n = 18$ ) are known of *C. × psammogena* so far. This might indicate an introduction of the hybrids from Europe, as triploid hybrids are extremely rare and sterile.

Key words: *Centaurea × psammogena* GÁYER, hybridization, distribution

### **Dynamic State Variable Model of Optimal Clutch Size in *Urophora affinis* (Diptera: Tephritidae) on Spotted Knapweed**

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A dynamic state variable model was developed for *Urophora affinis* (Frauenfeld)(Diptera: Tephritidae), an introduced biological control agent of spotted knapweed, *Centaurea maculosa*. The model predicts optimal clutch size (i.e., egg allocation) in *U. affinis* for the

maximization of fitness across different capitula sizes, and predicts the frequency distribution of the total number of capitula with 0, 1, 2,....., 11 galls per seed head. Model predictions are then compared to field data obtained from six spotted knapweed sites in Montana. The results of  $X^2$  Goodness of Fit tests showed that model predictions of the frequency distribution of seed heads with 0-11 galls per head agreed well with observed values in 4 of 6 sites, considered. In contrast predictions of the frequency distribution of galls across capitula of different diameters showed less agreement (one of six sites). However, Kolmogorov-Smirnov tests of predicted versus observed values for 1) the number of seed heads with 0-11 galls per head, and 2) the number of galls as a function of capitula diameter agreed well for all sites considered.

Key words: *Urophora affinis*, spotted knapweed, *Centaurea maculosa*, egg allocation, fitness, resource exploitation, behavioral ecology

### **New Foreign Explorations for Classical Biocontrol of Spotted Knapweed**

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Biocontrol of spotted knapweed (SK) in North America has a long history. Both on SK and diffuse knapweed, a total of thirteen seed head and root boring insects have been released during the last thirty years. It appears that success in reducing SK has not developed as expected.

Therefore, three reasons have emerged for new foreign explorations: 1) except for the root-boring moth, *Agapeta zoegana*, none of the insects released overtime have demonstrated a beneficial impact on reducing the spread of SK; 2) a recent molecular study on *Centaurea* species proved the Eastern Europe origin of SK introduced into the U.S. (Ochsmann 2000); 3) climatic similarity was shown between the western U.S., Romania and western Russia, Ukraine, and Turkey (Rice *et al.* 1999).

Among the thirteen released species, twelve originated from Greece and adjacent countries (Switzerland, Austria, Romania, Bulgaria, Hungary, ex-Yugoslavia); only the banded seedhead fly, *Urophora affinis*, originated from ex-USSR. We may hypothesize that plasticity of insect species originating in warm climates is not sufficient for permanent establishment in northwestern U.S. states. New biocontrol agents that are adapted to colder climates may allow more successful biological control of SK in northern areas. Association of climate matching and distribution of SK indicates favorable conditions for future foreign explorations above the 45<sup>th</sup> parallel in Eurasia. Research on SK will be resumed with explorations mainly in the former Soviet Union and Ukraine. These areas of origin of the target plant have not been extensively investigated before and could provide some specific new agents for our cooperators. There may be similar issues with *C. diffusa*, since diffuse and SK share so many biological control agents in common.

For a genetic study of SK, samples from diverse parts of Europe and the U.S. will be collected by scientists at Colorado State University.

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Key words: foreign exploration, Biocontrol, *Centaurea maculosa*, *Centaurea diffusa*, East Europe.

**Survival of the Root Mining Biological Control Agents *Agapeta zoegana* and *Cyphocleonus achates* in Spotted Knapweed Treated with Three Concentrations of the Herbicides Tordon and Transline**

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*Centaurea maculosa* (spotted knapweed) is an invasive alien plant that has become problematic in much of Northwestern North America. Over the last 50 years, techniques such as herbicide spraying and biological control have been implemented to control its spread. The herbicide Tordon (picloram) has been utilized throughout this period because of its effectiveness. Transline (clopyralid) is also an effective herbicide and is less toxic to trees than Tordon, however it is more expensive. Recently much effort has been placed on using biological control agents to reduce the use of these herbicides. This research explores whether or not herbicides and biological control insects can be used together on the same plants without detriment to the insects. We focused on the survival of 2 biological control agents, *Agapeta zoegana* and *Cyphocleonus achates*, in spotted knapweed plants that have been treated with low rates of the herbicides Tordon and Transline. Permanent plots were established in two areas that had low-density establishment of the two insects. The insect populations within the plots were augmented to ensure that relatively even numbers of insects would be present for each treatment. Treatments included controls and three rates of each of the herbicides. Each treatment was replicated 3 times at each site for a total of 42 treatment plots. Ten days were allowed for the herbicides to be translocated within the knapweed plant. Plots were destructively sampled to recover the larvae mining the knapweed roots. Each larva was rated as alive or dead. A Chi-square analysis revealed no significant difference ( $p=0.018$ ) in survival across all treatments for the Cramer Creek and Rock Creek study sites. This research indicates that within the confines of this study there is no effect of the herbicides on the insects.

Key words: herbicides, *Centaurea maculosa*

## **Spotted and Diffuse Knapweed – grazing and integrated management**

### **Sheep Grazing Spotted Knapweed**

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Spotted knapweed *Centaurea maculosa* displaces native semiarid grasses in the foothills and valleys in the Northern Rocky Mountain region, possibly because the dominant large domestic herbivores in this system, cattle and horses, avoid grazing spotted knapweed. As a small ruminant, sheep may help restore a balance to these infested plant communities by grazing spotted knapweed. In a series of studies, we assessed: 1) viability of spotted knapweed seed after passing through sheep and tame mule deer, 2) to what extent sheep graze spotted knapweed and associated plants, 3) effect of sheep grazing on infested plant communities, and 4) effect of spotted knapweed on rumen microorganisms. Following a pulse dose of seed, tame mule deer and sheep passed 11 and 4% of spotted knapweed seed, respectively. Sheep stopped passing seed after 4-5 days, whereas some deer were still passing seed after 10 days. Viability of passed seed was much lower than unfed seed, but some seed were still viable. Sheep readily graze spotted knapweed, but they also graze associated plants slightly more or less than spotted knapweed depending on plant phenology. Repeated, moderate sheep grazing had minor effects on native grasses and mature spotted knapweed plants, but reduced flower stem production and the presence of young spotted knapweed plants indicating that, in time, sheep grazing could reduce the presence of spotted knapweed. High levels of spotted knapweed in the 'diet' depressed rate and amount of microbial activity, explaining why sheep, like most herbivores, prefer a mixed diet. With prudent management, sheep have the potential to control spotted knapweed while using this invasive weed as a forage resource.

Key words: Spotted knapweed, *Centaurea maculosa*, seed, mule deer, native grasses, rumen microorganisms

## **Integrating 2,4-D and Sheep Grazing to Manage Spotted Knapweed Infested Rangeland**

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The objective of this study was to determine the effects of integrating 2,4-D and repeated sheep grazing on spotted knapweed (*Centaurea maculosa* Lam.) infested plant communities. Four treatments were replicated three times in a randomized-complete-block design at each site. The treatments were: 1) 2,4-D amine (2.1 kg ha<sup>-1</sup>) applied in the spring, 2) sheep grazing (95% knapweed utilization) repeated three times in 1998, 3) 2,4-D amine (2.1 kg ha<sup>-1</sup>) applied in the spring and sheep grazing (95% utilization) repeated three times in 1998, and 4) a control which did not receive 2,4-D or sheep grazing. Spotted knapweed density,

grass and spotted knapweed biomass, and percent cover of spotted knapweed, downy brome (*Bromus tectorum* L.), other grasses, litter and bare-ground were measured at peak standing crop in 1998. Data were analyzed using analysis of variance. Main effects of both sheep grazing and 2,4-D application lowered spotted knapweed seed head density and biomass. At Site 1, 2,4-D increased downy brome biomass and cover. However, when sheep grazing was combined with 2,4-D, downy brome biomass and cover were lowered from that of the 2,4-D alone. Sheep grazing alone had bare-ground and litter cover similar to that of the control, while plots treated with 2,4-D had higher bare-ground cover and lower litter cover.

Key words: sheep, grazing, *Bromus tectorum*

## **Grassland Restoration in the Weed Capital of the West: Changing Missoula Attitudes**

**Kate Supplee**

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Missoulians long have cherished nearby open spaces for their scenic beauty, recreational opportunities, and wildlife habitat. This appreciation, paired with good community planning, led to the passage of City open space bonds in 1980 and 1995. Bond acquisitions have added nearly 3,000 acres to Missoula's urban area open space system, which is augmented by 600 acres of University land and three nearby Lolo National Forest recreation areas.

Although the community was forward-thinking in its efforts to preserve open space, it was slow to understand the equal importance of good land stewardship. Public lands suffered from rapidly-growing infestations of knapweed, leafy spurge, dalmatian toadflax, and sulfur cinquefoil. But concern about herbicides led to political paralysis. Meanwhile the values for which the lands were acquired began to be severely impacted.

In the early 1990s public land managers, the Missoula County Extension Agency, and the County Weed Office grew increasingly concerned about the noxious weeds threat. They also recognized the requisite need for public education. They acted to compile an annual newspaper insert, install demonstration areas, talk with school groups, provide field trips, and develop vegetation plans for City and University open space lands.

The success of this public education effort can be measured by the June 2000 passage of a mill levy increase for noxious weed control in Missoula County. Attitudes are changing in the former Weed Capital of the West; we're now seeing broad support for "doing something about the weeds."

Key words: Missoula, open space, political

**Grassland Restoration in the Weed Capital of the West:  
The Importance of Education and Public Involvement**

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Social aspects of weed control can drastically affect a program's success. The City of Missoula and the University of MT are in the early stages of implementing an integrated vegetation management program for public natural areas. In order to move from arguing to making progress in weed control, education and public involvement are included at every stage of the program. The program includes "killing weeds," revegetation with native species, and monitoring of results to allow for adaptive management. The program emphasizes hands-on education for K-12 students and adults via participation in weed pulls, field trips and other restoration projects. After 2 years, we have successfully implemented several hundred acres of weed control, and there appears to be public and administrative support for sustenance of the program.

Key words: public, weed management

**Integrated Management of Spotted and Diffuse Knapweed**

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Spotted and diffuse knapweed (*Centaurea maculosa* and *C. diffusa*) are introduced species that infest millions of acres throughout the western United States and southwestern Canadian provinces. Both weeds can be effectively controlled with herbicides, however on some sites public perception and/or environmental conditions limit herbicide applications. The objective of this study was to determine the effectiveness and economics of various management techniques alone and in combination for knapweed control. Two upland range study sites were established on spotted knapweed in western Montana, and one study site was selected on diffuse knapweed in west-central Colorado. Treatments were applied to 20 by 30 foot or 10 by 30 foot plots arranged in randomized complete block design with 3 or 4 replications. Treatments included hand pulling, mowing, herbicides, and the root weevil *Cyphocleonus achates* alone and in combination. Percent vegetative cover and visual percent control data were collected for each treatment. Permanent transects were established and a point-frame, or quadrats utilized for data collection. The only increase in grass cover was with treatments that included herbicides. Tordon (Trademark) 22K, Curtail (Trademark), and Transline (Trademark) provided the most cost effective and efficacious control of the knapweeds over multiple years with the greatest increase in grass cover. Hand pulling twice for two consecutive years was the most expensive treatment providing 0 to 35% diffuse and

spotted knapweed control respectively after three seasons, and significantly increased bare ground. Mowing twice for two consecutive years in some cases increased knapweed cover. Insects alone and combined with herbicides may prove cost effective for long term management of knapweed if insects establish and maintain suppression of weed populations.

Key words: *Centaurea maculosa*, *Centaurea diffusa*, Integrated Weed Management

### **Controlling Spotted Knapweed through Selective Defoliation at Varying Phenologic Stages**

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Spotted knapweed (*Centaurea maculosa* Lam.) is a major rangeland weed problem in the western United States. Prescription grazing has been suggested as a viable, economic method of spotted knapweed control. A 3-year defoliation study was initiated in 1999 to assess whether grazing spotted knapweed infested rangelands is a viable control method. The study site was located about 11 km northwest of the USDA-ARS Sheep Experiment Station, near Dubois, Idaho. Defoliation treatments were based on the type of plant defoliated and spotted knapweed phenology at the time of simulated grazing. Spotted knapweed, other herbaceous vegetation, no vegetation, or all vegetation in each study plot was clipped to 3 cm when spotted knapweed plants were in the rosette, bolt, or flowering stage. There were 8 reps of each of the 12 combinations of phenology and defoliation for a total of 96 permanent 0.1 m<sup>2</sup> plots. Annual and perennial forbs, annual and perennial grasses, and spotted knapweed were counted and basal cover was recorded. Shrub canopy cover and spotted knapweed flowers per plot were also recorded. All variables, except for number of knapweed flowers were analyzed as the change between years. Defoliation of spotted knapweed, either alone or in combination with the associated vegetation significantly reduced ( $P=0.02$ ) spotted knapweed basal cover compared to the control plots and significantly reduced flower production ( $P<0.001$ ) in both years. The associated vegetation was largely unaffected by defoliation treatments. These data are preliminary but suggest that livestock grazing may be an effective management technique for controlling spotted knapweed.

Key words: Spotted knapweed, *Centaurea maculosa*, defoliation, phenology, grazing



## Potential Forage Value of Spotted Knapweed

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To consider using livestock for weed management, the perception of weeds must be converted to one of feeds. Our objective was to evaluate the forage value of spotted knapweed (*Centaurea maculosa* Lam.). We collected mature and immature plants from a sagebrush grassland site in southeastern Idaho between May and September of 1999 and 2000. In 2000, we separated the samples into leaf, stem, and flowering portions. We analyzed plants for neutral detergent fiber (NDF), acid detergent lignin (ADL), *in-vitro* dry matter digestibility (IVDMD), total non-structural carbohydrates (TNC), and crude protein (CP) and conducted a preference trial using sheep. Neutral detergent fiber was lowest in May (25%-35%) and increased throughout the season (53%-63% in September; excluding fall rosette with 28% NDF). Immature plants had less NDF throughout the season (28%-31% in May; 52%-55% in September) than large mature plants (32%-38% in May; 60%-65% in September). Rosette leaves had less NDF (25%-31%) than stems (50%-66%) throughout the season. Acid detergent lignin increased throughout the season from 4%-6% in May to 8%-10% in August, and there was surprisingly little difference between leaves and stems (7% compared to 8%, respectively). *In-vitro* dry matter digestibility decreased throughout the season ranging from 69%-73% in May to 40%-48% in September (excluding fall rosettes). Total non-structural carbohydrates concentrations were higher in June (22%-26%) and lowest in September (9%-10% excluding fall rosettes). Crude protein ranged from 3%-22%. When sheep were fed dried rosette, bolting, and flowering knapweed, they initially preferred rosettes but expressed no distinct preferences in following trials.

Key words: forage value, spotted knapweed, *Centaurea maculosa*, grazing, phenology

## Developing Prescription Grazing Guidelines for Controlling Spotted Knapweed with Sheep

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Spotted knapweed (*Centaurea maculosa* Lam.) is a perennial herbaceous plant that is considered one of the most troublesome rangeland weeds in the northwestern United States and Canada. Contemporary weed management focuses on herbicide and bio-control, yet overlooks livestock grazing in control strategies. A study was recently initiated to examine the potential of sheep grazing to control spotted knapweed. Grazing trials were conducted on private ranchland in eastern Idaho with 2-year-old dry ewes from the USDA-ARS Sheep Experiment Station near Dubois, Idaho. Two rangeland sites (22 ha each) were fenced and

divided into 21 paddocks. Sheep were grazed at 2 stocking rates for 4 days in the rosette, bolting, or flowering growth stages with appropriate ungrazed controls. In each paddock, 24 sampling plots (1830 cm<sup>2</sup>) were randomly placed and permanently marked along 3 transects. Spotted knapweed density and canopy cover of spotted knapweed, other forbs, grasses, shrubs, and bare ground were assessed before and after the growing season to track community changes. The number of flowers produced was also recorded in the post-season measurements to indicate reproductive potential. In the initial season of the study, grazing had little effect on canopy cover compared to controls, except there was a tendency for grazing to reduce cover of native forbs on one site. The number of flowers per plot was slightly lower in grazed paddocks particularly when grazing occurred during the rosette stage. The density of immature plants was slightly lower in paddocks grazed during the flowering stage than controls. Therefore, early season grazing may affect flower production, and grazing late may reduce density of young plants. Data from the first season of grazing can only show initial trends of treatment differences and effect on plant community dynamics.

Key words: spotted knapweed, *Centaurea maculosa*, prescription grazing, grazing management, sheep, herbivory

### **Salmon River Non-chemical Spotted Knapweed Control**

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The Salmon River watershed in northern California, largely federal land managed by the U.S. Forest Service, is home to three Federally listed species of fish, and a network of community members that would rather not see herbicides used in the watershed. Since 1997 when spotted knapweed was discovered in the river corridor, the Salmon River Restoration Council (SRRC), a community based non-profit corporation, launched a workforce to control the pest without the use of chemicals. The mission of the SRRC is to protect and restore the Salmon River ecosystems through diversification of the local economic base by focusing on restoration of anadromous fisheries resources, promoting cooperation and communication between resource managing agencies, and active participation of the local community. Techniques used to date have been hand pulling and digging, propane torching, mulching with black plastic, and mowing. Inventory to date has resulted in approximately 150 gross acres of infestations ranging from very dense (more than 10 plants per square foot), to single plants scattered along roadsides on 130 sites.

The U.S. Forest Service has completed an Environmental Assessment for the eradication of spotted knapweed in the watershed. The preferred Alternative is one developed in response to the community's concerns about chemical use, which allows non-chemical methods to be employed as long as criteria for the reduction of the infestations, seed set, and the expansion of sites are met. These effectiveness criteria are being applied to the sites with the densest infestations, and were developed by weed scientists from U.C. Davis. There is one year of data available. The next data will be available in May, 2001.

This poster illustrates the progress towards eradication on selected sites with photos taken before/during/and after certain treatments. The effectiveness criteria is displayed.

Key words: non-chemical, *Centaurea maculosa*, control, riparian areas

**Spotted and Diffuse Knapweed - detection and mapping**

**Detecting and Mapping Spotted Knapweed in Rangeland Ecosystems Using Airborne Digital Imagery**

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Remote sensing has the potential to provide rapid and cost-effective methods for detecting and mapping the spatial distribution and dispersal patterns of noxious weeds over large areas. A critical component of effective weed management involves detecting infestations early and preventing their spread. New airborne digital imaging systems provide high resolution, in both spatial and spectral scales, that might be necessary for accurate weed mapping. Our objective is to compare the relative benefits of various spatial and spectral resolutions for detecting and mapping spotted knapweed in Montana rangelands. Multispectral 3-band, 0.3 m and 4-band, 1 m imagery, and hyperspectral 128-band, 5 m imagery were acquired for the study site. The methodology of this research utilizes over 300 ground-truthed training sites to develop spectral signatures to differentiate target weeds from native vegetation, and assess the accuracy of the resulting maps. The spectral signatures will be used for supervised classifications using several algorithms, including maximum likelihood and spectral angle mapper. An error matrix will be used to assess the overall accuracy, errors of inclusion, and errors of exclusion. Preliminary classification of the 1 m imagery resulted in an overall accuracy of 60%. Increased spectral resolution of the hyperspectral imagery should increase overall accuracy, while the decreased spatial resolution (5 m) should have a countervailing effect. Methods for implementing remote sensing as a weed detection and mapping tool will be assessed based on accuracy and operability. Resource managers can then weigh the tradeoffs of these alternative imaging systems for achieving management objectives.

Key words: spotted knapweed, airborne digital imagery, spectral signatures, supervised classification

**Use of a Geographic Information System (GIS) for Monitoring Large-Scale Diffuse Knapweed Control Efforts at the Rocky Flats Environmental Technology Site**

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Weed-control monitoring is a critical component of any integrated weed management program. Rocky Flats Environmental Technology Site (Site) ecologists have been using a geographic information system (GIS - ArcView) for several years, in addition to quantitative monitoring, to assist with weed-control monitoring. GIS analyses have been used to document the distribution of weed species on the Site, assess the effectiveness of herbicide applications, evaluate the longevity of applications, and provide important planning

information for future weed control. Annual sitewide mapping of diffuse knapweed (*Centaurea diffusa*) distribution has been conducted since 1997. Weed distributions are classified into four density categories; scattered, low, medium, and high. Based on the 1998 map, diffuse knapweed occurred on 2900 acres of the approximately 6500 acres at the Site. In May 1999, 1500 acres of grassland at the Site were treated by helicopter with the herbicide Tordon 22K to control diffuse knapweed infestations at several locations. To track the effectiveness of herbicide applications, spray locations were entered into the GIS. Using the clip theme tool in ArcView™, results from GIS analyses showed that, within the treated areas, diffuse knapweed levels were reduced by an average of 78% across all density categories during the first season after application. The greatest reductions occurred in the high (-91%) and medium (-83%) categories. After two years, overall diffuse knapweed levels remained at 45% below their original levels within the treated areas, with the greatest reductions still occurring in the high (-76%) and medium (-52%) categories. The scattered and low categories have shown increases as diffuse knapweed slowly returns. Additionally, mapping data revealed areas and terrain where less effective control has been achieved. This information, along with other quantitative data, is proving useful for improving weed control efforts at the Site.

Key words: diffuse knapweed, GIS, Rocky Flats

### **Sampling and Modeling Invasive Plant Infestations: Techniques for Identifying Plant Distribution in Rangeland Environments**

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Mapping plant distribution change over time is essential for successful management. It is also critical for measuring management success. The evaluation of invasive plant management efforts requires repeated, delineated maps that are time consuming and costly to collect. In order to better assess invasive management strategies, quicker and more efficient methods of mapping are necessary. This study examined different spatial sampling and distribution modeling techniques as a means to decrease the time and money necessary for rangeland invasive plant mapping. The specific objectives for the study were to 1) use interpolation modeling to test sampling methods and sampling sizes for identifying plant distribution and 2) determine an optimum invasive plant sampling strategy. Twelve hundred hectares in southwestern Montana were mapped for spotted knapweed. Three sampling methods (random, systematic, random-systematic), using six different percentages of the survey area sampled (0.04%, 0.06%, 0.08%, 0.11%, .16%, .25%) were applied to the data set. An inverse-distance weighting (IDW) interpolation modeling technique was used in ArcView GIS 3.1 to create spotted knapweed distribution maps from the samples. Results show that systematic sampling is consistently better at predicting spotted knapweed distributions than the other sampling methods. Resulting distribution maps also indicate that USGS map accuracy standards can be met using IDW interpolation techniques when at least 0.16% of the study area is sampled

Key words: interpolation modeling, plant distributions, mapping, *Centaurea maculosa*

## **Yellow Starthistle**

### **Comparison of North American and Eurasian Yellow Starthistle Populations using AFLP Fragment Pattern Analysis**

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Yellow starthistle (YST), *Centaurea solstitialis* L., (Compositae, Carduinae) is an invasive annual weed of rangeland, natural areas and disturbed sites, primarily concentrated in the western U.S. YST was first reported in the U.S. in California in the mid 19th century, probably arriving as a contaminant in alfalfa or shipping ballast. An efficient colonizer, YST now infests over 10 M ha in California, with a genetically complex population structure suggestive of multiple introduction events. A clarification of the population structures and genetic complexity of North American YST will facilitate the development of strategies for biological control of the weed in ecologically diverse sites. Identification of the foreign geographic origins of North American YST populations by matching of weed genotypes is a primary objective of this research, and is expected to aid in the exploration for biocontrol pathogens and insects colonizing foreign YST populations most similar genetically to their North American relatives. To accomplish this, we are using automated fluorescent Amplified Fragment Length Polymorphism (AFLP) DNA fragment pattern analysis to compare genotypes of North American YST with those of Eurasian YST populations and other allopatric *Centaurea* spp. collected from areas surrounding the Mediterranean, Black and Caspian Seas. Results from pairwise DNA fragment binary scoring similarity analyses will be presented, comparing AFLP patterns from North America and Eurasian YST populations.

Key words: Yellow starthistle, *Centaurea solstitialis*, invasive weed, biological control, biocontrol, AFLP, DNA fragment pattern

### **A Landscape Strategy for the Management and Control of Yellow Starthistle in the Salmon River Canyon**

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Yellow starthistle has been spreading south in the rugged canyon of the lower Salmon River drainage over the past 25 years. Past management approaches have not slowed the spread of this invasive weed. Recently a landscape strategy was implemented to contain the spread of yellow starthistle. The broad scale approach incorporates multi-agency/landowner weed

management activities into one integrated program. This integrated program relies on a series of management elements that include continuous inventory, specific species objectives and priorities, management zones, tactical treatment lines, planned follow-up, rehabilitation of weed dominated lands, local test plots, organizational focus and diligence, while taking into consideration the relationship of yellow starthistle to habitat conditions and other invasive weeds. These elements will be reviewed as well as operational changes that had to take place to effectively implement a broad-scale interagency strategy. Progress in containing the spread of yellow starthistle in the lower Salmon River Canyon as a result of the management strategy will be summarized.

Key words: *Centaurea solstitialis*, management strategy

### **Clientele Behavioral Changes Necessary for Effective Yellow Starthistle Management Strategies**

**Carl Crabtree**

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Many technical, science-based strategies have been developed and used successfully in management of yellow starthistle, and other weeds. However, these strategies will have limited long-term success until people change their behavior in relation to weed management. Idaho County Weed Control, is using new approaches to "people based weed management", through the Salmon River Weed Management Area. People are now developing ownership of the weed problems in the area. With that ownership, they have also "owned" the solutions, and are implementing them. Recent behavioral changes as a result of broad-scale weed management strategies, will be discussed in relation to past approaches.

Key words: *Centaurea solstitialis*, management

### **Ability of Annual and Perennial Grass Communities to Withstand Invasion by Yellow Starthistle**

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Plant communities differ in their ability to withstand invasion, dependent on successional stage and competitiveness. Yellow starthistle (*Centaurea solstitialis*) density and frequency were monitored in islands of bluebunch wheatgrass (*Pseudoroegneria spicata*), intermediate wheatgrass (*Thinopyrum intermedium*), Idaho fescue (*Festuca idahoensis*) and sheep fescue (*Festuca ovina*) dominated communities and their surrounding annual grass communities. The two fescue communities were least susceptible to invasion and bluebunch wheatgrass was the most susceptible perennial grass dominated community. Annual grass communities were most susceptible to invasion by yellow starthistle. Patchy distribution of yellow

starthistle seedlings was observed in areas that produced high biomass in the previous year. This patchy distribution possibly resulted from a secondary effect of competition, namely litter deposition. Subsequent greenhouse study results of litter effects on yellow starthistle germination determined that lower light intensity rather than changes in light quality suppressed germination. Yellow starthistle was susceptible to competition from perennial grass dominated communities. In addition, litter depth appeared to play a role in suppression of yellow starthistle in both annual and perennial plant dominated communities.

Key words: Yellow starthistle, *Centaurea solstitialis*, bluebunch wheatgrass, *Pseudoroegneria spicata*, intermediate wheatgrass, *Thinopyrum intermedium*, Idaho fescue, *Festuca idahoensis*, sheep fescue, *Festuca ovina*, competition, germination

### **A Novel Genetic Approach to the Control of Noxious Weed Populations: The Seed Arrest System (SAS) for Control of Yellow Starthistle in Western US Rangelands**

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Yellow starthistle (*Centaurea solstitialis*) has been recognized since the 1920s as noxious weed in the Pacific Northwest. Infestations are estimated at over 8 million acre, primarily in California, Oregon, Washington, Idaho, with smaller populations in Nevada, Arizona, Utah and New Mexico are currently infested with yellow starthistle. Yellow starthistle invades rangeland, pastures and roadsides, competes with native plant communities, and reduces biological diversity and the value of grazing land. As an annual, yellow starthistle relies exclusively on seeds for reproduction, nearly all of which are produced by outcrossing. Rangeland plants may average 10,000 to 40,000 seeds per m<sup>2</sup>, most of which germinates or is lost to predation or decay within three years. We propose to develop a new strategy for the control of Yellow starthistle based on a genetic system developed to prevent the spread of transgenes from crops to wild relatives. We call this strategy the Seed Arrest System (SAS). The basic concept is similar to the use of sterile males in the biological control of insect pest populations. Our proposal is to produce transgenic starthistle plants that contain a controllable gene system that will, when activated by chemical treatment of seed, grow to normal size and appearance. The plants however will not produce seed but will produce pollen that carries a seed development arrest gene. This pollen competes with non transgenic pollen in fertilization of wild starthistle flowers. Flowers fertilized with transgenic pollen do not make seed but continue to compete with fertile plants for resources. Thus fertile plants also produce fewer seeds, further decreasing overall seed output, a significant factor for reducing yellow starthistle populations to establish more desirable vegetative cover.

Key words: yellow starthistle, *Centaurea solstitialis*, noxious weed control, rangeland, genetics



## **The Complexities of Grazing Management Following Yellow Starthistle Control and Wheatgrass Establishment**

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Much of the research devoted to management of (*Centaurea solstitialis*) on rangelands has focused on two important areas: 1) control techniques for plant communities dominated by the invader; and 2) revegetation attempts to convert degraded annual communities to perennial grasses either during or post-yellow starthistle control. However, the relationships between grazing management, post-treatment (*Centaurea*) control, and perennial grass establishment are largely uncharacterized. In April 2000, we initiated a study to compare simulated grazing at different phenological stages of yellow starthistle development, in a series of plots previously planted with pubescent wheatgrass in 1997 and treated for 1, 2, or 3 years with clopyralid. The same grazing treatments were also applied to a series of plots that previously received 1, 2, or 3 annual clopyralid applications without wheatgrass being planted. Simulated, high intensity, short duration grazing was done with a sickle-bar mower at a height of approximately 10 cm. The simulated grazing dates corresponded to three phenological stages of yellow starthistle development: rosette (April 1), mid bolting (May 15), and early flowering (June 15). Yellow starthistle recovery (cover, density, biomass, and seedhead production) was assessed in August.

Factorial analyses indicated highly significant ( $p < 0.001$ ) main effects and interactions among all factors for yellow starthistle cover, biomass, density and seedheads produced. A simulated graze during either the bolting or early flowering stage generally resulted in decreased yellow starthistle recovery compared to the control. However, simulated grazing during the rosette stage had either no effect or increased yellow starthistle recovery compared to the controls. Yellow starthistle generally increased in wheatgrass plots grazed in the rosette stage. However the trend was generally not significant. Wheatgrass recovery will be assessed in mid-November. These first year results indicate that yellow starthistle management strategies may strongly interact with post treatment grazing management.

Key words: *Centaurea solstitialis*, pubescent wheatgrass, grazing management

### **A Positive Experience in Weed Management: an Overview of the Tri-State Demonstration Weed Management Area**

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In 1996, the Bureau of Land Management funded four Demonstration Weed Management Areas to promote innovative thinking in the way weeds are managed. Tri-State was one of these groups. Cooperators developed strategy to fit the existing weed challenges and focused on cooperative management across boundaries. Through five years of working together, cooperators have developed an atmosphere of enthusiastic sharing and information transfer.

This open exchange has facilitated advances in weed knowledge, treatment techniques, biological control methods, and rehabilitation efforts. Along the way Tri-state has been able to support advances in remote sensing of weeds through hyperspectral imagery, aid in the development of nursery sites and subsequent distribution of biocontrol agents through cooperative field days, and make use of the cooperators' past experiences to develop site treatment prescriptions most likely to succeed in rehabilitation efforts. One of the strong points of the group is an ability to respect the various viewpoints of participants and understand the limitations or restrictions placed on some by regulation or mission. The strength of Tri-State is in the diversity of participants. As the group moves from a demonstration area into the long term, we are faced with the inevitable shift in budgets, personnel, and focus that must be a part of any dynamic entity. In addition, recent wildfires have provided innumerable opportunities and challenges in assessing weed spread and effect to biocontrol promotion.

Key words: weed management

### **Seed Germination of Yellow Starthistle and Spotted Knapweed after Treatment with Picloram or Clopyralid**

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Four trials were established to determine the seed germination potential after herbicide treatments. Treatments were applied at the same 3 timings for both weed species: rosette, bud and flower. On yellow starthistle and in 1 spotted knapweed trial treatments were: picloram at 0.125, 0.25, 0.375 lb/A; picloram + 2,4-D at 0.125 + 1.0, 0.25 + 1.0, 0.375 + 1.0 lb/A and an untreated control. In the other spotted knapweed trial treatments were: clopyralid at 0.125, 0.25, and 0.375 lb/A; clopyralid + 2,4-D at 0.125 + 1.0, 0.25 + 1.0, and 0.375 + 1.0 lb/A, and untreated control. At the rosette stage only the picloram alone treatments were applied. Seeds were collected 1 week after full flowering for spotted knapweed and 2 weeks after full flowering for yellow starthistle. Yellow starthistle seed viability was significantly reduced with all treatments over the untreated controls at the bud and flower stage. At the bud stage the addition of 2,4-D significantly reduced viable seeds only with 0.125 lb/A rate. At the flower stage, seed viability was reduced 16-35% over untreated plants even though plants did not appear to be controlled and flowering was not reduced. The most effective treatments for seed viability reduction (90-100%) were applications made at the bud stage, except picloram at 0.125 lb/A. No spotted knapweed seeds were formed at bud stage treatments except picloram at 0.125 lb/A and clopyralid at 0.125 lb/A. Seeds were 6 and 23% viable, respectively. Flower stage treatments did not appear to control plants but all rates of both products did significantly reduce the viability of seeds produced. At flower stage, seed viability was reduced 52-76% by picloram and 52-

64% by clopyralid over the untreated control seed. Bud stage was the most effective timing for seed viability reduction

Key words: *Centaurea solstitialis*, *Centaurea maculosa*, seed germination

## **Reproductive Phenology in Yellow Starthistle**

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Timing of reproduction in relation to soil moisture and competing vegetation strongly influences seed output, a crucial factor in population dynamics of annual invaders. In the Pacific Northwest, yellow starthistle reproductive phenology is conspicuously later than associated vegetation, including other Mediterranean invaders, which avoid summer drought by early flowering and senescence. A study to investigate the influence of temperature, photoperiod and intraspecific competition on yellow starthistle reproduction used germination at constant temperatures to establish a base temperature. A yellow starthistle population from Lewiston, Idaho, was established at successive planting dates and four densities near Moscow, Idaho (46.77° N. Lat). A thermal time model with a base temperature of 2°C adequately predicted reproductive phenology in this population. Cohorts emerging between October and July required about 1240 degree days for 50% of the plants to reach bud stage, and an additional 500 and 900 degree days to anthesis and achene dispersal, respectively. Considerable variation within cohorts was observed on either side of the 50% level in the population, (first and last plant at each stage). Competition for moisture delayed, rather than hastened, development. Under weather conditions in Moscow, Idaho, no post-bud stage plants from any planting survived the winters. Insensitivity to photoperiod and lack of a vernalization requirement allow spring germinating plants to reproduce if moisture is adequate.

Key words: phenology, reproduction, thermal time model, yellow starthistle, *Centaurea solstitialis*

## **Effects of Defoliation on Reproduction of Yellow Starthistle**

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Yellow starthistle (*Centaurea solstitialis* L.) is an introduced Asteraceae that has become established on 10 million acres in the Pacific Northwest and California. This weed functions as an annual or short-lived perennial and depends on seeds for reproduction. Strategies of plant control that reduce plant fitness or lower seed production or viability may help limit the

rate of spread of yellow starthistle. Previous work has shown that grazing and mowing can influence seed production. We tested the hypothesis that timing and frequency of defoliation can reduce the number and viability of seeds produced. Our study was conducted in Umatilla County, Oregon using a randomized block design with 4 replications of each of 4 treatments: (1) non-defoliated control; (2) single defoliation at the bolting stage; (3) single defoliation at the floral bud stage; (4) two defoliations, once at the bolting stage and again at the floral bud stage. Each of 4 blocks consisted of a 12 x 12 m area, with 16 plots measuring 3 x 3 m. Plants were defoliated at ground level using a gas-powered string-type mower. Response measurements were collected at the end of the growing season (September) following potential regrowth and included: (1) number of seedheads/plant; (2) number of seeds/seedhead; (3) number of seeds/plant (plumed and non-plumed); (4) number of seeds/m<sup>2</sup>; (5) seed viability (% germination); and associated plant and environmental statistics (plant height, number of branches/plant, soil moisture, populations of 5 biological control insects species). The treatment most effectively reducing seed production (seedheads/plant, seeds/plant, seeds/m<sup>2</sup>) and plant fitness (plant height, number of branches) was defoliation at the bolting stage and again at the floral bud stage. There was no statistical difference in percent germination among treatments. Defoliation had no effect on the infestation rates of seedheads by biological control insects. We conclude that the seed production of yellow starthistle can be reduced significantly by grazing or mowing to ground level during the bolting stage and again during the floral bud stage, and such treatment is compatible with the use of biological control insects.

Key words: Yellow starthistle, *Centaurea solstitialis*, grazing, defoliation

### ***Chaetorellia succinea* – Is this Unintentionally Released Natural Enemy of Yellow Starthistle Safe?**

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During 1996, we detected *Chaetorellia succinea* damaging the flower heads of yellow starthistle in California and Oregon (Balciunas and Villegas 1999). We determined that this fly had unintentionally been released in Oregon, in a shipment of *Chaetorellia australis*, an approved yellow starthistle agent, that had been contaminated with this similar-appearing fly. Earlier, in brief investigations in Europe, *C. succinea* had been rejected as a potential agent for yellow starthistle because of concern that it might develop on safflower, *Carthamus tinctorius* (Sobhian and Zwölfer 1985).

We, therefore, immediately began field and laboratory investigations of this fly's host range. We planted safflower at several sites where this fly was abundant, and monitored safflower fields at 47 sites in California. We also collected heads from almost 20 varieties of *Cirsium* and *Centaurea* at field sites in California and Oregon. We held these heads in our laboratory, and determined if *C. succinea* emerged from any of them.

These field surveys and tests were supplemented by laboratory host specificity tests. We exposed *C. succinea* to five varieties of safflower, under both choice and no-choice conditions, and monitored the test plants for emergence. We also tested, under no-choice conditions, another 10 species in the thistle tribe for their acceptability as hosts for this fly.

In the lab, none of the three species of *Cirsium* we tested was accepted as a host, and likewise, in the field none of the heads we collected from 15 varieties of this genus ever produced *C. succinea*. One of the four species of *Centaurea* that we evaluated in the lab allowed a few *C. succinea* to develop, and our colleagues at California Department of Food and Agriculture (CDFA) have detected this fly in the field on this and several other weedy *Centaurea* species.

More troubling was the fact that under no-choice conditions, and sometimes even in our choice tests, oviposition and eventual emergence was observed on nearly all of the safflower varieties we tested. Although none of the "trap" safflower we grew at field sites with large populations of this fly showed any damage from this fly, we did eventually detect the presence of *C. succinea* at two of the 47 safflower fields we monitored. At one of these fields, a population of *C. succinea* has persisted for several years on safflower. The damage to safflower at this field from *C. succinea* was not very great.

This fly is now widespread in California, and has established in at least four other states. Some of the *C. succinea* populations in California are quite large, and this fly may contribute to controlling yellow starthistle at some sites. However, this fly is not an approved agent, and because of the low risk of damage to commercial safflower growers, we do not recommend deliberate introductions of *C. succinea* to other areas.

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Key words: Biological control, *Centaurea solstitialis*, false peacock fly

### **State-County Distribution of Three Biological Control Agents of Yellow Starthistle in California**

**Baldo Villegas, Dale Woods, Mike Pitcairn, Don Joley and John Gendron**

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Three biological control agents of yellow starthistle, the bud weevil, *Bangasternus orientalis* (Capiomont), hairy weevil, *Eustenopus villosus* (Boheman), and the gall fly, *Urophora sirunaseva* (Hering) have become widely established in California. The bioagents were part of a statewide distribution program for weed biological control agents implemented by the California Department of Food and Agriculture's Biological Control Program. The distribution program was set up in 1988 with the California County Agricultural Commissioners and Sealers Association (CACASA). The goal of the program was to

improve the efficiency of dissemination of biological control agents throughout California and to promote the use of biological controls as alternatives to other forms of pest control. The program requires the active participation of biologists from county agriculture departments and other government agencies within the state of California. Workshops are conducted at centralized field nursery sites and are designed to train the participants in the identification, collection and release of the biological control agents. Participants collected available biological control agents, then returned to their own sites and released the bioagents in order to establish their own nursery sites for further distribution. Followup surveys of some of the releases were required until establishment had been achieved. Thereafter, the county agencies collected from their own nursery sites for in-county redistributions or participated further in the CDFA sponsored workshops.

## **Squarrose Knapweed and Other Species**

### **Importance and Distribution of *Centaurea* Species in Turkey**

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The flora of Turkey is one of the richest floras in the world because of its geographical position. Turkey also consists of three different floristic regions (Euro-Siberian, Irano-Turanian, and Mediterranean). According to 1998 data, the total number of species in the Turkish flora is 8800 (8576 of them native). While 8000 weed species are problematic in the world, over 1500 weeds are recorded in Turkey. Among them, Genus *Centaurea* is widespread and includes 172 species. Nearly 20 *Centaurea* species cause economic damage in cultivated land, pastures, field margins, road sides, orchards and nurseries in Turkey. Some crop seeds such as wheat and anise also contain *Centaurea* seeds. *Centaurea solstitialis*, *C. calcitrapa*, *C. cyanus*, *C. depressa*, *C. triumphetti*, *C. carduiformis*, *C. iberica* and *C. virgata* are the dominant knapweed species. Most knapweed research has focused on their distribution and density, but little is known about their management in Turkey. *Centaurea* weeds are recently more important in pasture areas in Turkey. The main reason is that they are spiny and nonedible to livestock. Their coverage is increasing year by year. Pastures in the east Mediterranean Region of Anatolia were surveyed for all weeds including *Centaurea* genus in our earlier studies. Investigations on biological control of *C. solstitialis* with emphasis on *Ceratapion basicorne* are in progress in the middle and south Turkey.

**Key words:** Knapweed, *Centaurea solstitialis*, *Centaurea calcitrapa*, *Centaurea cyanus*, *Centaurea depressa*, *Centaurea triumphetti*, *Centaurea carduiformis*, *Centaurea iberica* and *Centaurea virgata*, survey, Turkey

### **Squarrose Knapweed: Occurrence and Natural History in Rangelands of Central Utah**

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Squarrose knapweed (*Centaurea virgata*) is a taprooted perennial generally developing a tight canopy of profusely branched stems up to 1 meter in height and width. It may occur as individual plants scattered among various vegetation types or as solid stands. In Central Utah it occurs on over 120,000 acres and threatens invasion into all community types of the Great Basin. Much of the life history of this plant is unknown and must be documented to allow land managers the best success at reducing the weed to acceptable levels. Some aspects of life history that are currently under study include seed production, seed germination requirements, seed dispersal, seedbank longevity, plant phenology and morphology, rates of population spread, population age structure and identification of community types susceptible to invasion. As information is gained in these areas it will be

incorporated into the next stage of studies which focus on species specific plant competition. In conjunction with herbicide application and biological control methods, we expect to identify an assemblage of species which individually or in combination can be used to restrict the spread and density of squarrose knapweed.

Key words: Squarrose knapweed, *Centaurea virgata*, Utah

## **Control of Squarrose Knapweed on Burned and Non-burned Rangeland**

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Picloram plus 2,4-D and clopyralid plus 2,4-D were applied October 23, 1996 to squarrose knapweed infestations on burned and non-burned rangeland. Knapweed in burned plots had not re-emerged following an August, 1996, wildfire. Approximately half of the knapweed plants in non-burned plots were in the seed head stage, and about 50 percent were seedling and rosette plants. Plots were evaluated visually in 1997, 1998, 1999, and 2000. Biomass dry weight yields of knapweed and grass were harvested from subplots in 1998 and 1999. Knapweed control in burned plots, averaged over all rates of picloram plus 2,4-D from 1998 through 2000, was 97 percent, compared to 16 percent control in plots that had not been burned. The same relationship was observed for the clopyralid plus 2,4-D treatment, with burned plots averaging 73 percent and non-burned plots averaging only 9 percent during the same 3-year time period. Light to moderate grass injury from picloram plus 2,4-D was evident for up to 1 year following application. However, grasses recovered quickly and yields were significantly higher in plots where knapweed control was greatest. The average dry-weight yield of grass in the picloram plus 2,4-D burned plots during 1998 and 1999 was 3070 kg per hectare, compared with 525 kg per hectare in the corresponding non-burned plots. Fire in the absence of herbicides appeared to promote the production of knapweed. In the burned non-treated check plots during 1998 and 1999, the average knapweed yield was 1.9 times greater than in non-burned plots.

Key words: *Centaurea squarrosa*, fire, burn



## **Russian Knapweed**

### **BASF Product Update for Knapweed Control**

**Joseph G. Vollmer and Jennifer L. Vollmer**

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Several BASF products have shown activity on knapweed species (*Centaurea* spp., *Acroptilon repens* (L.) DC.). Products have been tested across the west at several application rates and timings. BASF has a product portfolio currently being developed for invasive weed control and renovation of non-crop, wildland and pasture/rangeland sites. These products include imazapic (Plateau), imazapic plus 2,4-D (Oasis), quinclorac, and diflufenzopyr combinations with dicamba and quinclorac. Each product has a fit for knapweed control.

Imazapic and the imazapic premix with 2,4-D have both shown excellent control of Russian knapweed (*Acroptilon repens* (L.) DC.) with a fall application at 0.188 lbs ai/A or 0.188+0.375 lbs ai respectively. Spring and summer applications of imazapic or imazapic plus 2,4-D to Russian knapweed have given inconsistent control. Diffuse knapweed (*Centaurea diffusa* Lam.) control with imazapic plus 2,4-D or dicamba plus diflufenzopyr was equal to picloram plus 2,4-D. For diffuse knapweed control, applications were made in spring to the rosette.

Imazapic, dicamba plus diflufenzopyr, quinclorac and quinclorac plus diflufenzopyr have been tested for spotted knapweed (*Centaurea maculosa* Lam.) control by Dr. Rod Lym at North Dakota State University. Dr. Lym found quinclorac plus diflufenzopyr gave the greatest control of 85% at 12 months after treatment and 73% control at 24 months after treatment. In this same trial, dicamba alone gave similar control to the quinclorac combination with diflufenzopyr. Quinclorac alone and the dicamba combination with diflufenzopyr gave control of less than 51%.

Spotted knapweed control with imazapic has been poor. However, imazapic offers cheatgrass (*Bromus secalinus* L.) and downy brome (*Bromus tectorum* L.) control when applied in combination with products applied for control of spotted knapweed. Areas infested with spotted knapweed often have an understory or seed bank of a *Bromus* species. Addition of imazapic to a spotted knapweed control program will stop a successive population of bromus. This allows for revegetation with desirable species, either through reseeding or release of species previously dominating the site.

Key words: imazapic, 2,4-D, quinclorac, diflufenzopyr, Russian knapweed, diffuse knapweed, spotted knapweed, cheatgrass, downy brome, Plateau, Oasis, *Acroptilon repens*, *Centaurea diffusa*, *Centaurea maculosa*, *Bromus* spp., *Bromus tectorum*, *Bromus secalinus*, herbicide

## **Factors that Make Russian Knapweed a Highly Competitive Plant**

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Previous studies have shown a competitive advantage by Russian knapweed, *Acroptilon repens* (L.) in other plant communities. Observations suggest allelopathy and a competitive adaptability that lead to its success in a variety of environments including precipitation zones, soil types, neighboring plant species, plant densities, fertility levels and other stress inducing factors. The competitive advantage continued to occur unless the weed was suppressed long enough by herbicides to allow the introduction and establishment of improved grass species. The determined success of either monoculture, knapweed or grass, is primarily attributed to a combination of allelopathy, moisture, nutrients and study duration. After 7 to 9 years following herbicide applications and planting of improved grass species, four sites were evaluated across varied precipitation zones having from 12.7 to 22.86 cm annual rainfall. Noticeably, there were few knapweed seedlings, indicating most all knapweed plants originated from lateral roots 7 to 14 cm below the soil surface. The competitive monoculture of Russian wildrye cv. 'Bozoisky' with its dense fibrous root system suggest the ability to capture needed plant moisture and nutrients, exude allelopathic compounds and reduce the root rhizosphere inhibiting entry of the knapweed lateral roots contributing to a successful monoculture compared with other grasses evaluated. Schrow suggests, 'A competitive advantage may exist by grasses when competing with a tap rooted plant for moisture and nutrients.' Study duration and the long time span required for evaluation can contribute to the determined success of either Russian knapweed or grass community dominance.

Key words: allelopathy, competitive, fibrous, Russian knapweed, *Acroptilon repens*, Russian wildrye

## **Precipitation Effects on Russian Knapweed Dominance**

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Environmental conditions, such as soil texture and precipitation levels, can affect the ability of individual species to dominate vegetative communities. Determining the response of a weed to environmental conditions may help us predict which lands are susceptible to future severe infestations. Russian knapweed (*Acroptilon repens*) is a long-lived perennial that affects rangeland throughout western North America. Observations in Colorado, USA, suggest that level of vegetative dominance by Russian knapweed may be related to

precipitation level, with lower precipitation areas having denser stands. We established a 3-year precipitation manipulation study at two sites in SE Colorado with *Bouteloua sp.* as the dominant grass and several distinct patches of Russian knapweed. Seasonal rainout shelters were constructed to alter precipitation levels on 3x3m plots. Treatments are: drought with shelter, control with shelter, control without shelter, and wet without shelter, receiving 0.5x, 1.0x, 1.0x, and 2.0x of natural May-September precipitation levels respectively for 3 consecutive years. Treatments were replicated 4 times at each site. The sites differ in soil texture. Russian knapweed cover and density were not significantly affected by treatment; however, site and year were significant factors. The differences between sites supports the hypothesis that higher clay soils are more likely to have dense knapweed infestations. The significance of year illustrates the natural variability in plant growth that makes determining influential environmental conditions difficult under field situations. We observed that, compared to control treatments, knapweed in wet and both shelter treatments tended to green up earlier and senesce later. Grass cover may have been affected by the precipitation treatments, the wet treatment having higher grass cover. Pending funding, this experiment will continue for several more years. More significant treatment differences may appear in the future since both the native grass species and non-native invader are long-lived and may be resistant to short-term manipulations.

Key words: Russian knapweed, *Acroptilon repens*, precipitation, *Bouteloua*

### **Fall Applications of Picloram for Control of Russian Knapweed Prior to Reseeding Perennial Cool-season Grasses**

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Russian knapweed, *Acroptilon repens* (L.) DC., is a deep-rooted, allelopathic perennial that forms monocultures on disturbed sites. Russian knapweed reduces biodiversity of wildlife populations and prevents all seedling plants from growing near mature plants. All treatments that provide control must release competitive species in the understory or be followed by reseeded before long-term sustainable control can be achieved. When perennial grasses such as Western wheatgrass and blue grama were present in the understory, single applications of 1 qt (0.5 lb ai/A) of Tordon 22K (Picloram) provided excellent competition resulting in control of 85 percent eight years following application. When disturbed sites were treated at the same rate only annual weed populations followed the treatment. Disturbed sites that were treated had to be reseeded before long-term control with competition could be achieved. When applications of picloram at 0.25 and clopyralid were applied two times before seeding and once one year after seeding, grasses were successfully established and provided control five years after they were seeded.

Key words: Russian knapweed, *Acroptilon repens*, Picloram, perennial grasses

Table 1. Long-term control of Russian knapweed in areas having understories of blue grama and western wheatgrass.

| Treatment <sup>1</sup> | Rate     | 1991<br>% control <sup>2</sup> | 1998<br>% control <sup>3</sup> |
|------------------------|----------|--------------------------------|--------------------------------|
| Picloram               | .5       | 86                             | 77                             |
| Picloram + 2,4-D       | .5 + 2.0 | 85                             | 85                             |
| Picloram               | 1.0      | 97                             | 97                             |

<sup>1</sup> Herbicides were applied with a hand-held sprayer on Oct. 10, 1990.

<sup>2</sup> Evaluations were made Aug. 28, 1991.

<sup>3</sup> Evaluations were made Sept. 26, 1998.

Table 2. Long-term control of Russian knapweed with 0.25 lb picloram followed by establishment of cool-season grasses.

| Grass Species                | Picloram<br>(0.25 lb/A) <sup>1</sup> | Clopyralid<br>(.27 lb/A) | No herbicide |
|------------------------------|--------------------------------------|--------------------------|--------------|
| Russian wildrye <sup>2</sup> | 90 <sup>3</sup>                      | 81                       | 8            |
| Thickspike wheatgrass        | 83                                   | 69                       | 14           |
| Western wheatgrass           | 82                                   | 80                       | 5            |
| Streambank wheatgrass        | 82                                   | 88                       | 16           |

<sup>1</sup> Herbicides were applied as repeated treatments Oct. 1991, Aug. 1992 and Aug. 1994.

<sup>2</sup> Grasses were seeded Apr. 1992.

<sup>3</sup> Control percentage was based on 100 random points/treatment, July, 1997.

### Potential Host Range of two *Urophora* Flies and an Eriophyid Mite for the Biological Control of Russian Knapweed

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Russian knapweed, *Acroptilon repens* (L.) DC. (Asteraceae), is relatively free of insects and pathogens in North America. Biological control of this invasive weed in North America utilizing exotic organisms has been to date somewhat limited. Only the gall-inducing nematode, *Mesoanguina (Subanguina) picridis*, has been introduced and established in North America with limited success. Other organisms are being considered for biological control (see U. Schaffner et al., in this Proceedings). Host specificity testing has been initiated on two species of gall flies: *Urophora kasachstanica* and *Urophora xanthippe*, and an eriophyid mite, *Aceria sobhiani*. Both flies induce a lignified gall in the flower head; which reduces seed production. The mite feeds on the plant's leaves and stems causing distortions, stunting, and/or plant stress. For the *Urophora* flies we conducted no-choice oviposition tests. Larval development tests were conducted on those plants in which eggs were laid. Fifty-five plants

were tested against *U. kasachstanica* and 49 species against *U. xanthippe*. *Urophora kasachstanica* appears to be host specific to Russian knapweed. Although oviposition occurred in three other plant species, no larval development occurred. *Urophora xanthippe* oviposited in a slightly wider range of hosts. Larval development tests need to be repeated to determine if these are potential hosts. No-choice development tests were conducted for the mite. Thirty-two plant species have been tested against *A. sobhiani*. The mite appears to be restricted to the tribe Cardueae. Although Russian knapweed is the more suitable host, mites were able to develop on *Centaurea americana*, *Cynara cardunculus*, *C. scolymus*, and *Silybum marianum*. Additional testing is required on this eriophyid to determine its suitability as a biological control agent.

### **Using Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) to Match Natural Enemies to Russian Knapweed.**

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*Acroptilon repens* (L.) DC., Russian knapweed (RK), is an aggressive perennial that poses an economical and ecological threat to grazing lands in North America. The USDA-Animal Plant Health Inspection Service, CABI Bioscience, state, and local agencies are exploiting the genetic diversity of RK in the search for insect natural enemies of this weed. The goal of this research was to develop and use a tailored RAPD-PCR protocol to locate genetic markers for identifying populations of RK in the U.S. and Eurasia. Analysis of RK specimens (60 U.S., 4 Kazakhstan, 3 Turkey, and 4 Uzbekistan collections) with RAPD primers OPA-10 and OPC-04 (Operon Technologies) has indicated that 53 out of 60 U.S. populations may have originated from sites located in Uzbekistan and Kazakhstan. No U.S. populations shared RAPD patterns with Turkey for any of the primers used. The data suggest that exploration for biological control agents of RK should be directed to RK populations existing in Kazakhstan and Uzbekistan.

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