Proc. VII. Int. Symp. Biol. Contr. Weeds, 6-11 March 1988, Rome, Italy.
Delfosse, E.S. (ed.). Ist. Sper. Patol. Veg. (MAF), pp. 499 - 509 (1989).

Field Experiment with the European Knapweed Rust (*Puccinia jaceae*) on Safflower, Sweet Sultan and Bachelor's Button

S. Hasan,[1] P. Chaboudez[1] and K. Mortensen[2]

[1] CSIRO Biological Control Unit, 335 Avenue Abbé Paul Parguel, 34090 Montpellier, France
[2] Agriculture Canada, Research Station, Regina, Saskatchewan S4P 3A2, Canada

Abstract

The European knapweed rust (*Puccinia jaceae*) infected four safflower cultivars, sweet sultan (*Amberboa moschata*) and bachelor's button (*Centaurea cyanus*) in greenhouse host-specificity tests. Fully susceptible pustules developed on safflower cotyledons, but the first true leaves showed partial resistance and no infection occurred on the upper leaves. As safflower is an important crop in North America, it was considered necessary to study the susceptibility of *P. jaceae* in the field to make sure that it would not cause problems if released as a biological control agent in North America. A field experiment was carried out to show the effect of *P. jaceae* on five safflower cultivars, sweet sultan and bachelor's button in the Mediterranean region. The rust infected severely early stages of all safflower cultivars. Upper leaves, similar to the situation observed in glasshouse tests, became resistant, but rust infections were observed on flower parts of all safflower cultivars. Bachelor's button was found to be susceptible at all plant stages, whereas sweet sultan remained uninfected. The effect on safflower plant height and seed production appeared minimal, but further studies on the effect of *P. jaceae* infection on safflower are desirable.

Introduction

Diffuse knapweed, *Centaurea diffusa* Lam. (Asteraceae) is a herbaceous weed of European origin which is spreading rapidly in the dry grassland of western Canada and U.S.A. The weed has no forage value and under some conditions largely replaces the herbaceous vegetation of greater value. Biological control has been considered as a suitable method for reducing the population of diffuse knapweed. Several arthropod species have been introduced into Canada for this purpose. They have reduced seed production but have so far failed to control the weed. Additional biocontrol agents are needed and being investigated (Harris and Myers 1984).

The rust fungus *Puccinia jaceae* Otth (Uredinales) was collected on diffuse knapweed in eastern Europe (Watson *et al.* 1981). This macrocyclic and autoecious rust has been shown to be virulent to diffuse knapweed from 15 different localities in western Canada and north western U.S.A. indicating that diffuse knapweed all over North America should be susceptible to the rust (Watson and Alkhoury 1981). However, during host-specificity tests in the greenhouse, safflower, *Carthamus tinctorius* L. (Asteraceae), was infected in the seedling stage, though older plant stages were resistant (Watson and Alkhoury 1981). Mortensen (1985) tested 4 safflower cultivars, US-10, S208, Rehbein and Hartman, and all of these were infected by *P. jaceae* under greenhouse conditions. The infection was mostly restricted to the first four leaves, often producing small resistant type pustules surrounded by chlorotic or necrotic areas. Among other plants closely related to diffuse knapweed, infected with *P. jaceae* were sweet sultan, *Amberboa moschata* (L.) DC, and bachelor's button, *Cyanus segetum* (L.) Hill (= *Centaurea cyanus* L.)

To ensure that the rust would not cause problems if released in North America as a biological control agent against diffuse knapweed, it was necessary to study the susceptibility of safflower to *P. jaceae* in the field. This paper describes a field experiment carried out in Mediterranean climatic conditions at Montpellier with this aim in view.

Materials and Methods

Plants

Five cultivars of safflower, S208, 80B2793-2, Rehbein, Hartman (seeds supplied by J.W. Bergam, Montana Agricultural Experimental Station, Sidney, MT, U.S.A.) and Lesaf 34C (seeds supplied by Dr. H.-H. Mundel, Agriculture Canada, Research Station, Lethbridge, AB, Canada), and in addition, two cultivars of sweet sultan, a purple-pink flowered (cultivar unknown) and a yellow flowered (Suaveolens-yellow, Ball-Superior Ltd., Mississaga, ON, Canada), and bachelor's button (Polka Dot, Dominion Seed House, Georgetown, ON, Canada) were used. Diffuse knapweed seeds used were collected from infestations in British Columbia. The seeds were surface-sterilized with 0.6% sodium hypochlorite for three minutes, dried and treated with the fungicide maneb (ethylenebisdithiocarbamic acid) to eliminate any contamination, especially the seed-borne safflower rust (*Puccinia carthami* Cda.). The seeds were sown in mid-April 1985 in seven plots of 9 m^2, in six rows 4.5 m long and 30 cm apart. Plot 1 was divided into two parts, half was sown with the purple-pink coloured, and the other half with the yellow coloured, cultivar of sweet sultan. The plants were watered in the dry season using a flat perforated plastic tube laid on the ground with the holes facing down, to avoid loss of the rust spores from aerial watering.

Inoculation

Lyophylized and non-lyophylized uredospores of *P. jaceae* isolates R11 and R13h2 were used for inoculation. Inoculation was made about one month after sowing when the safflower plants had developed four to six leaves and sweet sultan and bachelor's button 2-3 leaves. Three pots of diffuse knapweed plants were transplanted into the second and fifth rows of each plot with the purpose of producing rust inoculum continuously throughout the growing season. Inoculation was made late in the afternoon to prevent inoculated plants from drying before natural dew formation took place. Uredospores of the two isolates of *P. jaceae* were mixed together and a water suspension of these was sprayed onto the plants in each experimental plot. Three potted plants of diffuse knapweed were also added to each plot at the time of inoculation. These were brought back into the greenhouse, incubated in humid chambers for 16 h and maintained in the greenhouse as controls for spore distribution and for the effectiveness of the field inoculation technique. Three additional healthy pots of diffuse knapweed plants were transplanted to each plot 15 d after inoculation in alternating rows with the inoculated diffuse knapweed plants. These served as controls for spore dispersal.

Spore Germination

Lyophylized uredospores were rehumidified by placing them in a growth chamber with 80% RH for 5 h before being used. Samples of lyophylized and non-lyophylized spores were seeded onto 2% agar plates which were stored at room temperature (20°C) for 24 h and then examined for spore germination. 400 spore counts were made to establish germination rate.

Post Inoculation Observations

Microscopic examination of inoculated leaves. Spore germination on the leaf surface and subsequent disease development in leaf tissue were followed using Bruzzese and Hasan's (1983) whole-leaf clearing and staining technique on two to three leaves of each inoculated species/cultivar, 2, 7 and 18 d after the field inoculation, the leaves then being examined under the light microscope.

Latent period. Latent period was determined as the number of days between the inoculation and time when the first pustules appeared on infected plants. Uredospores produced on plants other than diffuse knapweed were examined minutely and compared with those of *P. jaceae* to avoid confusion due to possible contamination with *P. carthami* or *P. centaureae*.

Disease assessment. Disease assessment was made by macroscopic examination of inoculated plants and by microscopic examination of cleared and stained leaves, from the time of inoculation onward, to follow the development of mycelium within the leaf tissue. Using these observations the resistance/susceptibility reactions of the test plants were rated according to the following scale of 0 to 9 infection types already used for other rust fungi (Line *et al.* 1974, Eskes and Toma-Braghini 1981).

0 Penetration of germ-tube occurs but there is no mycelial development in the host tissue. No visible sign of infection or symptoms.

1 Development of a few hyphae in between cells around stomata. Minute chlorotic and/or necrotic flecks hardly visible to naked eye. No sporulation.

2. Very restricted development of fungal mycelium occurs within the host tissue. Larger chlorotic and/or necrotic spots appear on the epidermis with no spore production.

3. Restricted growth of mycelium occurs within the host tissue. A mixture of various sizes of necrotic spots appears on the leaf surface. No uredospore production.

4. Less restricted growth of mycelium occurs within the host tissue and larger chlorotic and/or necrotic lesions appear on the epidermis with occasional development of small pustules with very few spores. Sporulation less than 25% of all lesions.

5. As in no. 4, but with more uredospore production. Sporulation <50% of all lesions.

6. As in no, 5 but with increased uredospore production. Sporulation <75% of all lesions.

7. As in no. 6 but with abundant uredospore production. Sporulation of up to 95% of all lesions.

8. A mixture of lesions with a varying degree of sporulation, often chlorosis behind sporulating area.

9. Only lesions with abundant sporulation, without marked chlorosis at the lesion border.

Plant height and biomass. Twenty mature, rusted plants per species/cultivar were selected at random and were measured from the base of the stem to the distal end. The mean height (with minimum and maximum) was calculated. These plants were cut at soil level and the aerial parts dried at 50°C for 4 d before being weighed to obtain their dry weight.

Seed production and their viability. Seed counts were made from all the flower heads available on 20 plants/species/cultivar. These were weighed to determine average seed weight of individual seeds. Germination was tested 1 wk later by placing them on damp filter paper in petri dishes maintained at 25±2°C.

Results

Spore Germination

The germination of lyophylized and non-lyophylized uredospores of *P. jaceae* received from Regina was tested before inoculating the test plants. Germination rates of lyophylized spores of isolates R11 and R13h2 were, respectively, 42% and 31% while those of non-lyophylized spores were 8% and 5%. To obtain sufficient spray-inoculum density, lyophylized and non-lyophylized spores from the two isolates were mixed together at the time of inoculation, giving 19% germination.

Microscopic Examination of Inoculated Leaves

Good spore germination and germ-tube penetration occurred on diffuse knapweed leaves examined after 2 d, followed by good mycelial development 7 and 18 d after inoculation. Markedly less mycelial growth was noticed on the leaves of the safflower cultivars and other species 2 and 7 d after inoculation. However, numerous infection sites were detected on leaves stained 18 d after inoculation in the five safflower cultivars and particularly in bachelor's button. Mycelial filaments seemed to grow normally, even penetrating the conducting tissue and forming well-developed haustoria.

Only on a few occasions was a localised cellular necrotic reaction noticed. In most infection sites, pustules developed with good sporulation, especially in bachelor's button. No germ tube penetration or mycelial growth was observed in the two cultivars of sweet sultan.

Latent Period

The seedlings of diffuse knapweed inoculated in the field at the same time as the test plants and incubated in the greenhouse as controls gave rise to the first visible pustules, usually on the lower leaf surface, 9 d after inoculation, while in the experimental plot this took 14 d. Safflower cultivar S208 produced pustules 16 d after inoculation while other cultivars and bachelor's button showed the first pustules 17 d after inoculation.

Disease Assessment

Disease assessments are given in Table 1. The following is a summary of the condition of the plants and nature of infections obtained:

Diffuse knapweed. All 21 inoculated plants of diffuse knapweed showed well developed pustules occasionally bordered by chlorotic flecks (Fig. 1A). The same number of control plants, inoculated in the greenhouse, gave rise to higher numbers of pustules with abundant spore production and without chlorotic flecks, (infection type 9). Pustules developed on both leaf surfaces, but more heavily on the lower surface.

Sweet sultan. The plants were well-developed with the formation of flower buds and flowers. None of the plants from either of the two cultivars showed any visible sign of infection.

Bachelor's button. The plants of this species were highly susceptible to the attack of knapweed rust and produced well-developed pustules with abundant spore production, especially on the lower surface of leaves, and with no chlorotic or necrotic lesions around the pustules.

Safflower cultivars. Rehbein - Good attack of the rust producing numerous pustules, up to 33/leaf, primarily on the lower leaf surface. In most cases, the pustules remained small and were surrounded by a yellowish halo. This latter aspect is in agreement with the microscopic examination of infected tissue where the mycelium was well developed with only occasional and localised cell lysis.

80B2793-2- Infection on this cultivar produced up to 34 small sporulating pustules, per leaf. All plants were attacked.

S208- Almost all plants had numerous small pustules, producing spores and surrounded by chlorotic or necrotic lesions (Fig. 1B).

Hartman - Practically all plants were infected, producing a large number of pustules with sporulation

Lesaf 34C - Heavy attack of the rust producing up to 65 small sporulating pustules on a leaf.

Table 1. Observations on plants in the field three weeks after inoculation with *Puccinia jaceae* Otth.

Parameter	Diffuse knapweed, (*Centaurea diffusa* Lam.), inoculated in		Sweet Sultan (*Amberboa moschata* [L.] DC.)	Bachelor's button (*Centaurea cyanus* L.)	Safflower (*Carthamus tinctorius* L.) cultivars				
	field	glasshouse			Hartman	Rehbein	Lesaf 34C	S208	80B2793-2
No. infected/ examined plants	21/21	21/21	0/71	51/79	83/86	74/80	82/84	84/91	86/89
Avg. no. leaves/plant									
Total	18	21	16.3	21.1	13	21.5	18	20	20.5
Infected	12	17	0	3.1	3.8	3.5	4.0	4.1	4.2
Avg. no. of pustules/leaf									
Minimum	9	> 100	0	1	1	1	2	1	1
Average	31	> 100	0	3	6	6	19	9	8
Maximum	54	> 100	0	12	17	33	65	35	34
Chlorotic/necrotic lesions	+	-	0	+	+	+	+	+	+
Infection type (0-9)	7	9	0	7	6	6	6	6	6

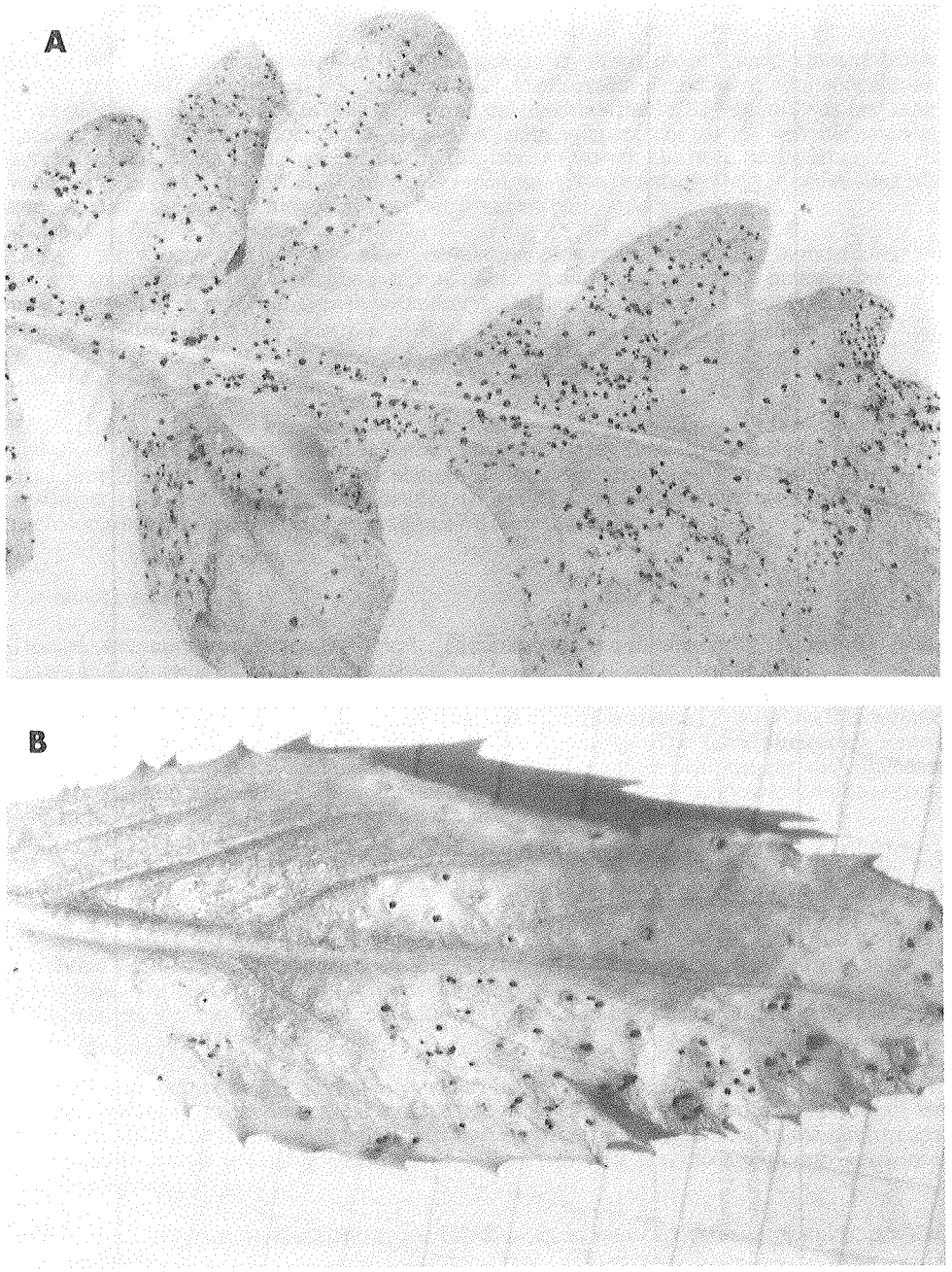

Figure 1. Uredosori of *Puccinia jaceae* Otth on: A. diffuse knapweed (*Centaurea diffusa* Lam.); and B. safflower (*Carthamus tinctorius* L.) cv. S208.

Four hundred uredospores from two pustules of each species/cultivar with rust symptoms were examined under the microscope and were confirmed as *P. jaceae*. All the spores observed had 2 germ pores. Most of the spores from test plants were also found to be viable, with a germination rate of 74 to 86%.

Observations on Test Plants Two Months After Inoculation

Leaf infection. Apart from sweet sultan almost all test plants developed rust pustules on their leaves, including new leaves developed later and absent at the time of inoculation (Table 2). Little difference was noticed among the degrees of infection of the five safflower cultivars. However, the rust pustules at this stage were small and less common than during the observations made 3 wks after inoculation. There was a noticeable increase of resistance with increasing height of the leaves, with the uppermost being totally resistant to rust infection. Uredospores from infected leaves were examined under the microscope and they were all found to belong to *P. jaceae.*

The average number of infected leaves/plant does not seem to be significantly different (Fig. 2). However, there were clearly lower numbers of pustules on the leaves of bachelor's button and still less on those of safflower cultivars, showing that the infection was far less severe on test plants than on diffuse knapweed.

As a precautionary measure, the stems of diffuse knapweed were destroyed, but all the rosette leaves were found to be well infected. The 21 healthy plants of the weed, which were introduced later into the plot were also equally infected, showing that there was good spore dispersal of the rust from one plant to another within the experimental plots.

Stem infection. In most of the test plants, stems did not show any rust infection, the exception being bachelor's button, which did develop a few rust pustules on the stem (Table 3).

Infection on flower parts. Bachelor's button had a few small pustules with chlorotic and necrotic lesions developed on the flowers (Table 4). About half the flowers on safflower cultivars showed chlorotic/necrotic lesions at their base with occasional small sporulating pustules. No major difference was noticed between the five safflower cultivars as regards the degree of rust infection.

Plant height and biomass. The heights of plants from the two cultivars of sweet sultan at the end of the season were found to be closely similar, as were those of the five safflower cultivars, while plants of bachelor's button were relatively taller. For all the test plants the dry weight biomass/plant was directly related to the heights.

Seed production. Seed production from the two cultivars of sweet sultan was approximately the same at the end of the season and less than that of bachelor's button. However, safflower seed production varied with cultivar, as might be expected, with a maximum of 120 seeds/plant for cv. 80B2793-2 and a minimum of 20 seeds for cv. Lesaf 34C. The weights of the seeds were proportional to their numbers.

Attempts made to germinate seeds from test plants were unsuccessful although kept for several days on moist filter paper. Seeds were examined and they appeared to be viable.

Discussion

It is evident from the above results that most of the test plants apart from sweet sultan, are susceptible to infection by the knapweed rust, bachelor's button more so than the safflower cultivars. Infection was more common on younger plants, producing relatively large numbers of pustules on the first few leaves. As the plants advanced in age the upper leaves became more resistant and total resistance developed in the uppermost leaves, similar to glasshouse tests (Mortensen 1985). Apparently the rust attack was not severe and did not seem to affect the plant growth, infected plants growing normally and giving rise to abundant flowers and seeds.

However, due to the slightly unexpected results obtained, this experiment was deficient in one important aspect. During the earlier greenhouse infectivity tests (Mortensen 1985), only a few pustules developed on the safflower cultivars. It was thus expected that a number of

Table 2. Evaluation of the leaf infection of test plants in the field two months after inoculation with *Puccinia jaceae* Otth.

Parameter	Diffuse knapweed (*Centaurea diffusa* Lam.)		Sweet Sultan (*Amberboa moschata*)	Bachelor's button (*Centaurea cyanus* L.)	Safflower (*Carthamus tinctorius* L.) cultivars				
	inoculated in field	not inoculated and transplanted later in the field			Hartman	Rehbein	Lesaf 34C	S208	80B2793-2
No. infected/ examined plants	21/21	21/21	0/11	20/20	20/20	20/20	20/20	20/20	20/20
Avg. no. of									
infected leaves/plant	26	21	0	22	13	17	11	14	14.5
pustules/infected leaf	30	24	0	14	1	1	1	1	1
Chlorotic (CL) and necrotic (NL) lesions	CL + NL	CL + NL	-	CL + NL	CL + NL	CL + NL	CL + NL	CL + NL	CL + NL
Infection type (0-9)	7	7	0	7	4	4	4	4	4

plants in the experimental plots at Montpellier would remain uninfected and could serve as controls, so that the effect of the rust infection on plant height, dry weight, and seed production of infected plants could be determined. Unfortunately this was not the case and all plants of bachelor's button and the five safflower cultivars were infected to some degree. Obviously it was difficult to estimate the significance of some of the results from infected plants in the absence of parallel data from healthy plants grown under the same conditions.

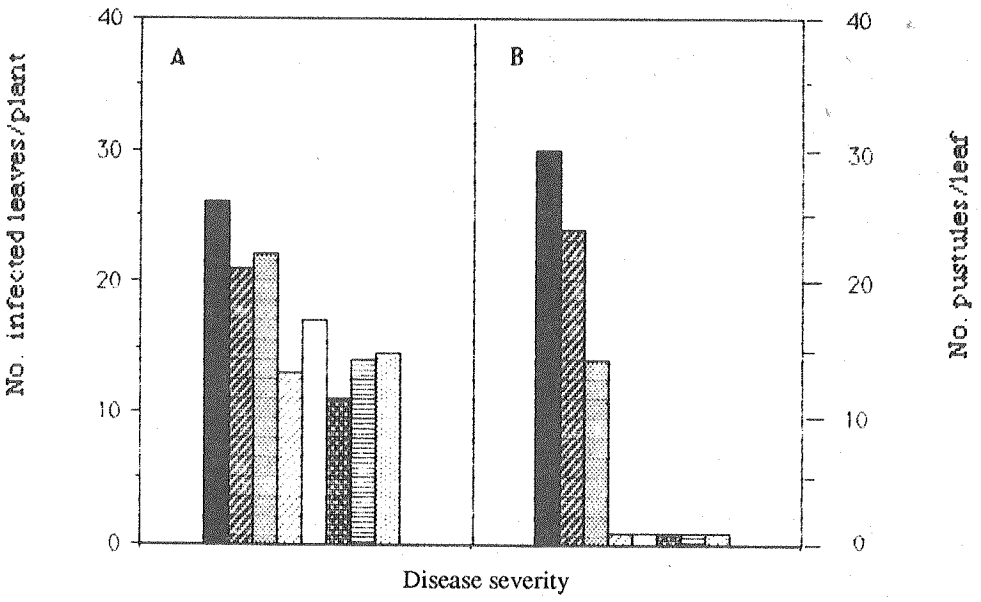

Figure 2. Disease severity of test plants two months after inoculation with *Puccinia jaceae* Otth. Bars on the left (A) show number of infected leaves; those on the right (B) give number of pustules formed/leaf. ■ Diffuse knapweed (*Centaurea diffusa* Lam.) inoculated in the field; ▨ diffuse knapweed not inoculated and later transplanted in the field; ▯ bachelor's button (*Centaurea cyanus* L.); safflower (*Carthamus tinctorius* L.) cultivars: ▨ Hartman, ▢ Rehbein, ▩ Lesaf 34C, ▤ S208 and ▦ 80B2793-2.

The results of the above experiment were complemented by observations made the following year on the carry over of the pathogen. The plots were left undisturbed with the remains of the dead plants, and in spring, spontaneous seedlings and planted seedlings of safflower were examined for any rust infection, which could have appeared as a result of spores persisting from the previous year's infected plants. None of these plants were found to be infected by *P. jaceae*. Thus it seems that occasional infection on safflower cultivars would not persist on the crops in subsequent years.

With the amount of infection observed on safflower in field tests, release *P. jaceae* in British Columbia, in an area that is relatively close to the important safflower growing area in Montana would be of some concern. It is felt that with the biological control agents (insects) already released and others currently being tested for biological control of diffuse knapweed in British Columbia, there could be sufficient control of this weed. Therefore, at this time studies on the rust will not be pursued further. However, further detailed work on its survival and its effect on safflower cultivars might prove that it would not be a threat to safflower production in North America.

Table 3. Infection of flowering shoots of field test plants two months after inoculation with *Puccinia jaceae* Otth.

Plant species/cultivar	Avg. no. infected plants/no. of plants examined	Avg. no. infected stems/avg. no. stems/plant	Avg. no. pustules/ infected stem	Chlorotic (CL)/ nectrotic (NL) lesions
Sweet Sultan (*Amberboa moschata* [L.] DC.)	0/11	0/ 7.8	0	0
Bachelor's button (*Centaurea cyanus* L.)	17/20	2.35/13.35	1.75	CL + NL
Safflower (*Carthamus tinctorius* L.) cultivars				
Hartman	0/20	0/ 4.45	0	0
Rehbein	0/20	0/ 6.95	0	0
Lesaf 34C	0/20	0/ 5.15	0	0
S208	0/20	0/ 6.00	0	0
80B2793-2	1/20	0/ 6.00	0	NL

Table 4. Infection of flowers of field test plants two months after inoculation with *Puccinia jaceae* Otth.

Plant species/cultivar	Avg. no. infected flowers/no. of flowers/plant	Avg. no. pustules/ infected flower	Chlorotic (CL)/ nectrotic (NL) lesions
Sweet Sultan (*Amberboa moschata* [L.] DC.)	0/ 7.7	0	0
Bachelor's button (*Centaurea cyanus* L.)	1.2/12.6	0.711	CL + NL
Safflower (*Carthamus tinctorius* L.) cultivars			
Hartman	2.25/ 4.45	0.456	CL + NL
Rehbein	3.00/ 6.75	1.433	CL + NL
Lesaf 34C	3.50/ 5.00	0.328	CL + NL
S208	2.00/ 6.00	0.244	CL + NL
80B2793-2	2.75/ 6.00	1.245	CL + NL

Acknowledgments

We would like to thank Drs. D.T. Briese, J.M. Cullen and P. Harris for carefully reviewing the manuscript and Dr. A. Sheppard for assistance with figures.

References

Bruzzese, E. and S. Hasan. 1983. A whole leaf clearing and staining technique for specificity studies of rust fungi. *Plant Path.* 32:335-8.

Eskes, A.B. and M. Toma-Braghini. 1981. Assessment method for resistance to coffee rust (Hemileia vastatrix) Berk. + Br.). *F.A.O. Plant Protec. Bull.* 29:56-66.

Harris, P. and J.H. Myers. 1984. *Centaurea diffusa* Lam. and *C. maculosa* Lam. s. lat., diffuse and spotted knapweed (Compositae). *In*: *Biological Control Programmes Against Insects and Weeds in Canada.* Kelleher, J.S. and M.A. Hulme (eds.). C.A.B., Slough, U.K., pp. 127-37.

Line, R.F., C.F. Konzac and R.E. Allan. 1974. Evaluating resistance to *Puccinia striiformis* in wheat. *Proc. Res. Co-ord. Meet., FAO/IAEA*, Novi Sad., IAEA Vienna, pp. 125-32.

Mortensen, K. 1985. Reaction of safflower cultivars to *Puccinia jaceae*, a potential biocontrol agent for diffuse knapweed. *Proc. VI Int. Symp. Biol. Control Weeds*, 19-25 August 1984, Vancouver, Canada. Delfosse, E.S. (ed.). Agric. Can., Ottawa, pp. 447-52.

Watson, A.K. and I. Alkhoury. 1981. Response of safflower cultivars to *Puccinia jaceae* collected from diffuse knapweed in eastern Europe. *Proc. V Int. Symp. Biol. Control Weeds*, 22-27 July 1980, Brisbane, Australia. Delfosse, E.S. (ed.). CSIRO, Melbourne, pp. 301-5.

Watson, A.K., D. Schroeder and I. Alkhoury. 1981. Collection of *Puccinia* species from diffuse knapweed in eastern Europe. *Can J. Plant Path.* 3: 6-8.