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Reaction of Safflower Cultivars to *Puccinia jaceae*, a Potential Biocontrol Agent for Diffuse Knapweed

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Abstract

Puccinia jaceae was tested on four safflower cultivars, US–10, S208, Rehbein, and Hartman, under growth chamber conditions. It was capable of infecting all four cultivars with fully susceptible pustules on the cotyledons. On the first set of true leaves, resistant-type pustules often surrounded by chlorotic and necrotic areas occurred, and no infections were observed on upper leaves from any of these cultivars. *P. jaceae* can be cultured on cotyledons of safflower, cultivar S208, for at least four generations without noticeable change in virulence on safflower as well as on diffuse knapweed. The effect of *P. jaceae* on development of safflower appears to be minimal under growth chamber conditions. The threat that this rust could pose to the safflower production, if released for biocontrol of diffuse knapweed, is discussed.

Réaction des Cultivars de Carthame a *Puccinia jaceae*, un Agent Biologique Potentiel de Lutte Contre la Centaurée Diffuse

Puccinia jaceae a été mise à l'essai sur quatre cultivars de carthames, soit US-10, S208, Rehbein et Hartman, dans des conditions de chambre climatisée. Elle a infesté les quatre cultivars et a provoqué la formation de pustules entièrement susceptibles sur les cotylédons. La première série de feuilles authentiques présentait des pustules résistantes, entourées de zones chlorotiques et nécrotiques, et aucune infestation n'a été observée sur les feuilles supérieures des quatre cultivars. *P. jaceae* peut subsister sur les cotylédons du cultivar S208 pendant au moins quatre générations sans modification notable de sa virulence à l'égard du carthame ou de la centaurée diffuse. L'auteur traite des risques que cette rouille pourrait faire courir à la production de carthame si elle était utilisée comme agent biologique de lutte contre la centaurée diffuse.

Introduction

Diffuse knapweed (*Centaurea diffusa* Lam.; Compositae) is a herbaceous weed of European origin which is spreading rapidly on the dry grasslands of western Canada and the U.S.A. (Watson and Renney 1974; Harris and Cranston 1979). Biological control is considered to be the most satisfactory solution, and several insects have been released and established in Canada for this purpose. They have inflicted damage, but not enough to control diffuse knapweed, and additional biocontrol agents are needed to increase the pressure on diffuse knapweed (Harris and Myers 1984).

Several rust species have been reported from *Centaurea* spp. in Europe (Gaümann 1959; Gayot 1967; Ialongo and Boldt 1977) and in 1978 a rust, *Puccinia jaceae* Otth. (Uredinales) was collected from diffuse knapweed in Eastern Europe (Watson *et al.* 1981). This rust was found to be virulent on diffuse knapweed collected from 15 different locations in western Canada and northwestern United States, indicating that diffuse knapweed populations in North America generally are highly susceptible to this rust

and that host plant resistance should not restrict its effectiveness as a biocontrol agent (Watson and Alkhoury 1981).

Tests under growth chamber conditions (Watson and Alkhoury 1981) revealed that *P. jaceae* was capable of infecting safflower (*Carthamus tinctorius* L.; Compositae) in the seedling stage, but later plant stages became resistant. Before release as a biocontrol agent on diffuse knapweed can be approved, it is essential to test its capability to develop on, and the effect, if any, it might have on safflower, a field crop cultivated relatively close to infested diffuse knapweed areas (Mortensen 1985).

The purpose of these experiments was to test susceptibility of safflower cultivars currently grown in Montana to *P. jaceae*, and the effect of the rust infections on safflower plants. The question of whether or not the rust can survive on safflower was tested by culturing it for successive generations of urediniospores on safflower cotyledons under growth chamber conditions.

Materials and Methods

European knapweed rust, *P. jaceae*, used in these studies was collected from diffuse knapweed in Rumania (Watson *et al.* 1981). Two isolates of this rust, R11 and R13h2, originating from single pustules on infected diffuse knapweed leaves that had been stored in liquid nitrogen for more than a year, were increased on diffuse knapweed in a growth chamber under quarantine conditions at Regina Research Station. Urediniospores used for inoculations were either freshly harvested from plants, or spores had been stored in glass vials in liquid nitrogen. Isolate designations refer to collection sites in Rumania (Watson *et al.* 1981).

Plants for the experiments were grown from seeds in plastic pots (15 cm diam.) in top soil (sandy-loam) mixed with sphagnum (3:1) under greenhouse conditions, temperature 18–24°C with a 16 h light period from 400 watt high pressure sodium lights. Seeds of safflower cultivars were obtained from safflower breeder, J.W. Bergman, Montana Agricultural Experiment Station, Sidney, Montana.

Plants were inoculated by atomization with a suspension of urediniospores in distilled water until runoff (concentration: approx. 0.5 million spores/ml). Inoculated plants were incubated in a dew-simulating chamber in total darkness for 18 h at 14 ± 1°C. After the dew period the plants were allowed to dry slowly before being placed in a growth chamber at 24 ± 2°C with a 16 h daily light period from cool flourescent and incandescent lights.

This study included three separate experiments:

Susceptibility test of safflower cultivars to P. jaceae. Five pots (five plants/pot) from each of four safflower cultivars, US–10, S208, Rehbein and Hartman, were inoculated separately with isolates R11 and R13h2 on 10–12 day-old seedlings, with cotyledons and the first set of true leaves developed. Infection type of pustules were recorded 14–16 days after inoculations. The experiment was repeated by inoculating three pots (2–3 plants/pot) from each cultivar with the two isolates at the 6–8 leaf stage (just prior to flowering). Infection type of pustules were recorded 17 days and again 45 days after inoculation. Control plants of inoculated diffuse knapweed plants were included with each test. The rating of infection type was done on a scale from 0 to 9:

0. Immune, no sign of infection;
1. Resistant, necrotic or chlorotic spots, no visible pustules;
2. Resistant, minute pustules (hardly visible) with very little sporulation, necrotic or chlorotic areas around pustules;

3. Moderately resistant, very small pustules (< 0.2 mm diam.) with little sporulation and chloratic areas around pustules;
4. Moderately resistant, small pustules (< 0.4 mm diam.) with some sporulation, chlorotic areas around pustules common;
5. Moderately susceptible, medium-sized pustules (< 0.6 mm diam.) with moderate sporulation, chlorotic areas around pustules occurs frequently;
6. Moderately susceptible, fair-sized pustules (< 0.8 mm diam.) with moderate to fair sporulation, slight chlorotic area around pustules can occur;
7. Susceptible, fair-sized pustules (< 1 mm diam.) with fair sporulation;
8. Susceptible, large pustules (< 1.3 mm diam.) with considerable sporulation; and
9. Highly susceptible, large pustules (> 1.3 mm diam.) with heavy sporulation.

Effectivity test of P. jaceae *on safflower.* Five pots of 19-day-old safflower (just prior to flowering) and five pots of 13-day-old plants (4–6 leaf stage when cotyledons were still in good condition) of cultivar S208 were inoculated with two isolates of *P. jaceae*, R11 and R13h2. Control plants for each age group and isolate were sprayed with distilled water. Control pots of diffuse knapweed were included with each test. Inoculated plants were rated for infection type 18 days after inoculation, reinoculated and rated again 16 days after the last inoculation. All plants were harvested 42 days after the first inoculation by cutting stems at the soil level and washing roots free of soil. Stems (including leaves and flower heads) and roots from each pot were dried for 24 h at 80°C and weighed separately. At harvest most plants from the young plant stage had completed flowering. Results were calculated as relative yield of dry matter in percent of controls.

Survival test of P. jaceae *on safflower.* Seedlings of safflower, cultivar S208, were inoculated with the two isolates, R11 and R13h2 (5 pots of 5 plants/isolate). Two wks after inoculation, infection type and density of pustules were recorded, then the urediniospores were harvested, suspended in water and inoculated on a new set of safflower seedlings and the plants from which the spores were harvested. This procedure was repeated four times, thus culturing the two isolates for four generations of urediniospores on safflower seedings.

Results

All four safflower cultivars tested were susceptible to the two isolates, R11 and R13h2, of *P. jaceae* on the cotyledons, but the first set of true leaves showed resistant reaction as indicated with infection types 7 or higher and 1–4, respectively (Table 1). The infection level on the cotyledons was high, with 50% or more of the leaf area covered with pustules, whereas, normally, only a few (1–5) developed on the first set of true leaves and very seldom were pustules observed on the second set of true leaves. Upper leaves were totally resistant to *P. jaceae*. Infected leaves senesced faster than uninfected leaves. As indicated in Table 2 it was possible to measure a slight reduction in yield of dry matter on infected safflower plants compared to uninfected plants. Safflower plants 19 days old at inoculation were least affected with little reduction in dry matter. Younger safflower plants (13 days old at inoculation) were affected more, especially the roots, where a 20% reduction in dry matter was observed for infected plants compared to uninfected safflower plants. No detectable difference was observed between the two isolates of *P. jaceae*, therefore, the results from both isolates were combined in Table 2.

The experiment to test the survival of *P. jaceae* through successive generations of urediniospores on safflower seedlings showed that both isolates could be successfully cultured on cotyledons of safflower for at least four generations without apparent loss in virulence on diffuse knapweed nor was increase in virulence on safflower detected.

Discussion

The data obtained in above experiments are in good agreement with data obtained by Watson and Alkhoury (1981), who tested two isolates of *P. jaceae* on six other cultivars of safflower. Based on these independent studies it can be concluded that safflower generally is susceptible to *P. jaceae* in the seedling stage, and that older safflower plants are resistant. Present studies show that safflower in the seedling stage is a satisfactory host for this rust to develop and reproduce on under growth chamber conditions, and also that safflower is slightly stressed when infected with *P. jaceae* in the early seedling stage.

Table 1. Infection types recorded on safflower cultivars and diffuse knapweed inoculated with *Puccinia jaceae* Otth. under growth chamber conditions.

Host plant	*P. jaceae* isolate	Infection type (scale: 0–9)[1]	
		Cotyledons	First set of true leaves
Carthamus tinctorius L.			
US-10	R11	8	2–3
	R13h2	7	2–4
S208	R11	7	1–3
	R13h2	7	1–4
Hartman	R11	7	1–4
	R13h2	7	1–3
Rehbein	R11	7	1–4
	R13h2	7	2–4
Centaurea diffusa Lam.	R11		9
	R13h2		8

[1]0 = immune; 9 = fully susceptible pustules.

It would be safe to assume that if infection can occur under growth chamber conditions, it can also occur under natural conditions. The next question is then, how likely will *P. jaceae* be exposed to safflower in the susceptible stage under natural conditions if released on diffuse knapweed in North America? Safflower is grown commercially on a considerable hectarage in central and eastern Montana and the Dakotas (Bergman *et al.* 1979). This is relatively close to diffuse knapweed infested areas in western Montana, Idaho and British Columbia. Thus it could be possible to have air-borne urediniospores from infected diffuse knapweed plants reach safflower crops. However, considering that safflower is only susceptible in the seedling stage, it is unlikely that the rust would be far enough advanced on diffuse knapweed in spring to produce a sufficient number of urediniospores that would allow spread to safflower crops while still susceptible. Should urediniospores happen to reach safflower seedlings, infections could occur, but chances that successive generations of urediniospores will develop on safflower within the susceptible stage is minimum. Thus, the rust has little chance of surviving on safflower. Since *P. jaceae*, according to Watson and Alkhoury

(1981) and unpublished studies conducted at the Regina Research Station, has potential for biocontrol of diffuse knapweed in North America, and since there is a need for additional biocontrol agents to suppress diffuse knapweed sufficiently in rangeland (Harris and Myers 1984), I feel that the slight chance that *P. jaceae* could pose a threat to safflower production might be worth taking in comparison to the added pressure this rust could inflict on diffuse knapweed.

Experiments will be conducted in France in 1985 to test *P. jaceae* on safflower cultivars under field conditions. If it does not survive on safflower through the season in these field tests, I feel that it will be safe to release *P. jaceae* on diffuse knapweed in North America.

Table 2. Yield in dry matter of safflower plants, cultivar S208, inoculated with *Puccinia jaceae* Otth. relative to non-inoculated plants.

Plant material	Age of plants at inoculation (days)	Relative yield (dry matter)[1] Control	Inoculated
Leaves and stems:	19	100	97.1
	13	100	92.5
Roots:	19	100	95.9
	13	100	79.7

[1]Plants were harvested 42 days after first inoculation (all plants had completed flowering). Results are based on data from two isolates of *P. jaceae* (average of 10 replications).

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References

Bergman, J.W., Hartman, G.P., Black, A.L., Brown, P.L., and Riveland, N.R. 1979. Safflower production guidelines. Capsule Information Series No. 8 (Revised), Montana Agric. Exp. Sta., Bozeman, MT, 25 p.

Gaümann, E. 1959. Beitrage zur Kryptogamenflora der Schweiz, Bd. XII. Die. Rostpilze Mitteleuropas. Buchler and Co., Bern, 1407 p.

Gayot, A.L. 1967. Les rouilles des Centaurees. *Uredineana* **6**: 59–161.

Harris, P., and Cranston, R. 1979. An economic evaluation of control methods for diffuse and spotted knapweed in western Canada. *Can. J. Plant Sci.* **59**: 375–82.

Harris, P., and Myers, J.H. 1984. *Centaurea diffusa* Lam. and *C. maculosa* Lam. s. lat., diffuse and spotted knapweed (Compositae). Ch. 31. *In*: Biological Control Programmes Against Insects and Weeds in Canada. Kelleher, J.S., and Hulme, M.A. (eds). Commonw. Agric. Bur., pp. 127–37.

Ialongo, M.T., and Boldt, P.E. 1977. Preliminary study on host specificity of *Puccinia centaureae* DC. on *Centaurea sphaerocephala* L. (in Italian). Extracts from Annali dell'Instituto Sperimentale per la Patologia Vegetale. **5**: 3–7.

Mortensen, K. 1985. A proposal for a standardized scale of attack and its application to biocontrol agents of weeds in laboratory screening tests. Proc. VI Int. Symp. Biol. Contr. Weeds, August 19–25 1984, Vancouver, Canada. Delfosse, E.S. (ed.) Agric. Can. (*in press*).

Watson, A.K., and Renney, A.J. 1974. The biology of Canadian weeds. 6. *Centaurea diffusa* and *C. maculosa*. *Can. J. Plant Sci.* **54**: 687–701.

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

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