EVALUATION OF *PUCCINIA CENTAUREAE* DC. AS A BIOLOGICAL CONTROL AGENT OF SPOTTED KNAPWEED (*CENTAUREA MACULOSA* LAM.).

by

Michel Clément

A thesis presented to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science

Department of Plant Science
Macdonald Campus of
McGill University
Montreal

 September 1984

Short title

PUCCINIA CENTAUREAE DC. ON SPOTTED KNAPWEED

MICHEL CLÉMENT

TO MY PARENTS

ABSTRACT

M. Sc. MICHEL CLÉMENT Plant Science

EVALUATION OF PUCCINIA CENTAUREAE DC. AS A BIOLOGICAL CONTROL AGENT OF SPOTTED KNAPWEED (CENTAUREA MACULOSA LAM).

Spotted knapweed is a noxious introduced weed, difficult to control with chemical, cultural, or managerial methods. One species of autoecious rust fungi collected on spotted knapweed was investigated as biological control agent. A total of 106 rust collections was made during a survey in Eastern and Central Europe in the summer of 1982. The material was sent to the plant quarantine facility of Macdonald College. Forty-eight rust collections were virulent on North American spotted knapweed. The most virulent rust isolate collected in Romania, identified as Puccinia centaureae DC., was able to infect 25 Centaurea species and also Amberboa moschata (L.)DC., Carthamus tinctorius L., and Cnicus benedictus L. Different levels of resistance were observed in many safflower cultivars. Three spotted knapweed rust collections did not differ in virulence to five safflower cultivars. Morphological studies showed appreciable differences in urediniospore shape and ornamentation of P. centaureae, P. jaceae Otth, and P. carthami Cda.

SOMMAIRE

M.Sc. MICHEL CLÉMENT Plant Science

ÉVALUATION D' URIDINÉES COMME AGENT DE LUTTE BIOLOGIQUE DE LA CENTAURÉE MACULÉE (CENTAUREA MACULOSA LAM.).

La centaurée maculée est une mauvaise herbe introduite d'Eurasie difficile à réprimer par les méthodes chimiques et culturales. Une rouille autoïque s'attaquant à cette plante en Europe a été évaluée comme agent de lutte biologique. Un total de 106 échantillons de rouille ont été prélevés sur la centaurée maculée en Europe Centrale et de l'Est durant l'été 1982. Les specimens ont été envoyés au centre de quarantaine du Collège Macdonald. Quarante-huit échantillons de rouille se sont avérés virulents sur la centaurée maculée de L'Amérique du Nord. L'isolat le plus virulent, provenant de Roumanie et identifié Puccinia centaureae DC., a aussi infecté 25 autres espèces de Centaurea ainsi qu' Amberboa moschata (L.)DC., Carthamus tinctorius L., et Cnicus benedictus L.. Divers niveaux de résistance ont été identifiés chez le carthame (Carthamus tinctorius). Aucune différence au niveau de la virulence a été observée entre trois isolats de rouilles inoculés sur cinq cultivars de carthame. L' étude de la morphologie des urédiniospores a permis de déceler des différences entre P. centaureae, P. jaceae Otth, et P. carthami Cda.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Alan Watson for his continued guidance and encouragement during the course of this study, and for critically reviewing the manuscript. I am especially grateful to Dr. Watson for having chosen me to conduct the collection trip in Europe in June 1982. The cooperation and interest shown, during the collection trip, by Dr. Dieter Schroeder from the Commonwealth Institute of Biological Control, in Delemont, Switzerland, is greatly acknowledged.

Sincere gratitude is extended to Ms. Colleen Cranch, Mr. Andre Virly, and Mr. Daniel Cloutier for technical assistance and unlimited collaboration. I wish to extend my thanks to my sister, Johanne, for typing tables of the manuscript. I wish to extend my appreciation to Dr. D.J.I. Buszard for thesis editing.

Postgraduate scholarship and research grants from the Natural Sciences and Engineering Research Council of Canada were greatly appreciated. Funds for the plant quarantine facility of Macdonald College through research agreements with the U.S.D.A., Biological Control of Weeds Laboratory, in Albany, California, were also greatly appreciated.

Finally, I wish to extend my thanks to Monic Côté, my family and friends for continued encouragement during this study.

TABLE OF CONTENTS

EXPERIMENTATION

LIST OF TABLES

LIST OF FIGURES

CHAPTER I. INTRODUCTION

Biological weed control may be defined as the deliberate use of natural enemies to reduce weed density to tolerable levels (Huffaker 1957). The recent progress and level of interest in biological weed control suggest that this strategy be now considered as an important component of weed control programs (Andres et al. 1976; Batra 1982; Schroeder 1983). This method is most attractive for weeds showing resistance or tolerance to herbicides or in cases where the costs of chemical control is prohibitive.

Although phytophagous insects have mainly been used in biological weed control (Anon. 1968), there has been an increasing interest in the use of plant pathogens as biocontrol agents of weeds (Freeman et al. 1978; Hasan 1980; Inman 1971; Templeton and Smith 1977; Wilson 1969). Strategy for biological weed control with plant pathogens includes a classic and a bioherbicides tactic (Templeton and TeBeest 1979). The bioherbicides tactic utilizes microorganisms as herbicide through mass inoculation on the target weed in a manner similar to chemical herbicides . The classic tactic involves the importation and release of exotic plant pathogens on alien weeds, where the control of the target weed is dependent upon the self-perpetuation and natural dispersal of

the pathogen. This tactic is more suitable for control of perennial weeds in areas such as rangelands, waterways and forests (Templeton and Smith 1977). Procedures in the classic tactic are; 1. determining the suitability of the target weed for biological control; 2. foreign surveys; 3. selection of suitable and effective agents; 4. host specificity tests; 5. release and establishment of the agent; 6. evaluation of efficacy of the agent (Harris 1971).

A program was initiated in 1965 for the biological control of spotted knapweed (Centaurea maculosa Lam.) (Zwölfer 1965). This weed is suitable for the classic tactic of biological weed control because it is an introduced perennial plant species from Eurasia forming extensive infestations in rangelands and pastures of western North America and is difficult to control by chemical, cultural and managerial methods (Harris and Cranston 1979). Four phytophagous insects were introduced on spotted knapweed in North America, but only two seed-head gall flies Urophora affinis Frfld. and U. quadrifasciata (Meig.) have established successfully (Harris 1980a, b). Although both flies spread rapidly and reduced seed production of spotted knapweed, they have not reduced the density of the weed to a satisfactory level, and other biocontrol agents will be required for successful control (Harris 1980a, b; Maddox 1982).

Rust fungi have proven to be effective and safe biocontrol agents of weeds (Hasan 1972, 1974a; Oehrens 1977).

Puccinia chondrillina Bubak & Syd., an autoecious rust fungus imported from Italy for the biological control of skeletonweed (Chondrilla juncea L.) in Australia, caused a significant reduction in the density of skeletonweed shortly after it was released (Burdon et al. 1981; Hasan 1972, 1974a). Virulent strains of P. chondrillina were also introduced and released in western United States where the rust became established and rapidly spread to uninoculated areas (Emge et al. 1981).

In its native range, spotted knapweed is also attack by autoecious, macrocyclic Puccinia rust species which have demonstrated a certain level of host specialization (Gaumann 1959; Guyot 1967; Jacky 1899). These rusts are suitable for investigation as possible biocontrol agents of spotted knapweed in North America.

The first objective of this study was to collect viable urediniospores of rust fungi attacking spotted knapweed in its native range and to import this material into Canada for further study in quarantine facilities. The second objective was to determine the pathogenicity of the rust collections on North American spotted knapweed, select the most virulent isolate and conduct host specificity tests. Finally, as a complementary study, the taxonomic position of the spotted knapweed rusts was determined in connection with morphological features of urediniospores and host range studies.

CHAPTER II. BIOLOGY OF SPOTTED KNAPWEED

2.1 NAME

Centaurea maculosa Lam.---spotted knapweed (Canada Weed Committee 1969); centaurée maculée, centaurée tachetée (Ferron and Cayouette 1964)--- is a member of the sub-tribe Centaurinae Dumort in the Cynareae Cass. tribe of the Asteraceae family (Dittrich 1977). It belongs to a complex and not well differentiated group of species of the sub-genus Acrolophus, section Maculosae (Dostal 1976). The systematics of this species group is not clear and the use of rather plastic characters in defining taxonomic units has led to the description of many infraspecific taxa by various European botanists. Consequently, taxonomic uncertainties concerning the European form(s) of C. maculosa exists in the literature.

Some authors use the prior name C. stoebe L., which may or may not apply (Beldie 1977; Hayek 1931). Rouy and Camus (1901) treated C. maculosa and seven other species as subspecies of C. paniculata L.. Hegi (1912) described three European subspecies of C. maculosa Lam.; ssp. eu-maculosa Gugler; ssp. rhenana Bor. (= C. stoebe= C. paniculata); ssp. micranthos (Gmel.)Gugler (= C. micranthos Gmel.). In Flora

U.S.S.R., these subspecies are raised to species level and only C. rhenana Bor. and C. micranthos Gmel. are described in the section Maculosae (Klokov et al. 1963). Dostal (1976) recognized four subspecies of C. maculosa Lam.; ssp. chaubardii (Reicherb. fil.) Dostal; ssp. albida (Lecoq and Lamothe) Dostal; ssp. subalbida (Jordan) Dostal; ssp. maculosa (= C. stoebe ssp. maculosa (Lam.) Hayek). He also considers C. biebersteinii DC. and C. rhenana Bor. as distinct species.

2.2 Description and variation of the weed

Spotted knapweed is a biennial or short-lived perennial herb 30-100 cm high. Stems are erect, ridged, pubescent, corymbosely branched, each branch bearing a single head. Basal leaves are deeply and irregularly pinnatifid, 2-3 times segmented or if not, linear. Leaves are canescent on both sides. Heads are discoid, 16-20 mm high, around 6 mm diameter. The involucre is 9-12 mm high, 6-8 mm broad, and ovoid. Phyllaries are ovate to ovate-lanceolate with a blackish apical fringe of five stiff processes 0.5-2.0 mm long. Flowers are tubular , purple, rarely white; marginal flowers sterile, ray-like ; central flowers perfect; achenes 3.0 mm long; pappus white, persistent, 1-2 mm long (Moore and Frankton 1974; Watson and Renney 1974).

The chromosome complement of the North American

spotted knapweed is reported to be tetraploid with 2n=36 (Moore and Frankton 1954). According to Guinochet (1957), the european C. maculosa s. str. has a chromosome number of 2n=18. Skalinska et al. (1959) reported a chromosome complement of 2n=18+(0-2B) for C. rhenana Bor. (= C. maculosa Lam. ssp. rhenana (Bor.)Gugler).

Guinochet and Foissac (1962) identified a tetraploid C. micranthos Gmel. (=C. biebersteinii DC. ssp. biebersteinii) at Cluj botanical Garden, Romania. A personal visit to the Garden in 1982 revealed that those plants did not have any characteristic features that would place it in the Maculosae section. Backsay (1958) reported a tetraploid C. biebersteinii in Hungary but no herbarium collections of this tetraploid form are known.

Moore (1972) distinguished North American populations of spotted knapweed with the ssp. micranthos (Gmel.) Gugler having small heads (involucre 10-11 mm high) and few (4-6 pairs) and short phyllary processes with black or brown margins. The ssp. rhenana (Bor.)Gugler and ssp. maculosa are distinguished by larger heads (involucre 11-14 mm) and 5-10 pairs of longer marginal, processes, which are black-dark brown in ssp. rhenana and partially white in ssp. maculosa.

Taxonomic studies on European and North American collections of spotted knapweed are underway at Macdonald College of McGill University to determine the relationship

between the tetraploid form(s) present in North America and the diploid form(s) that exists in Europe. Preliminary cytological studies have revealed that tetraploid form(s) are also present in Europe (Hungary and Romania) and are morphologically similar to the North American weed populations (A.K. Watson, pers.comm.). In studies conducted in Europe at the Commonwealth Institute of Biological Control, Delemont, Switzerland, it was observed that the North American spotted knapweed could be a tetraploid form of C. maculosa ssp. micranthos, but such a form has not yet been found in Europe (D. Schroeder, pers. comm.).

Because of the paucity of in depth taxonomic studies on the Norh American spotted knapweed, conclusions about its relationship with the European form(s) are difficult to determine. Attempts to do so are confounded by the description of many closely related species and subspecies which intergrade morphologically and geographically in Europe. It is therefore proposed that the European populations of spotted knapweed, on which field studies were conducted, be treated as a complex. This C. maculosa complex would include the three subspecies as described earlier by Hegi (1912).

Although Moore (1972) described three subspecies of C. maculosa in North America, it is still not known if the North American populations of spotted knapweed are composed of tetraploid or/and diploid plants. Studies are underway at Macdonald College to determine if morphological and genetical

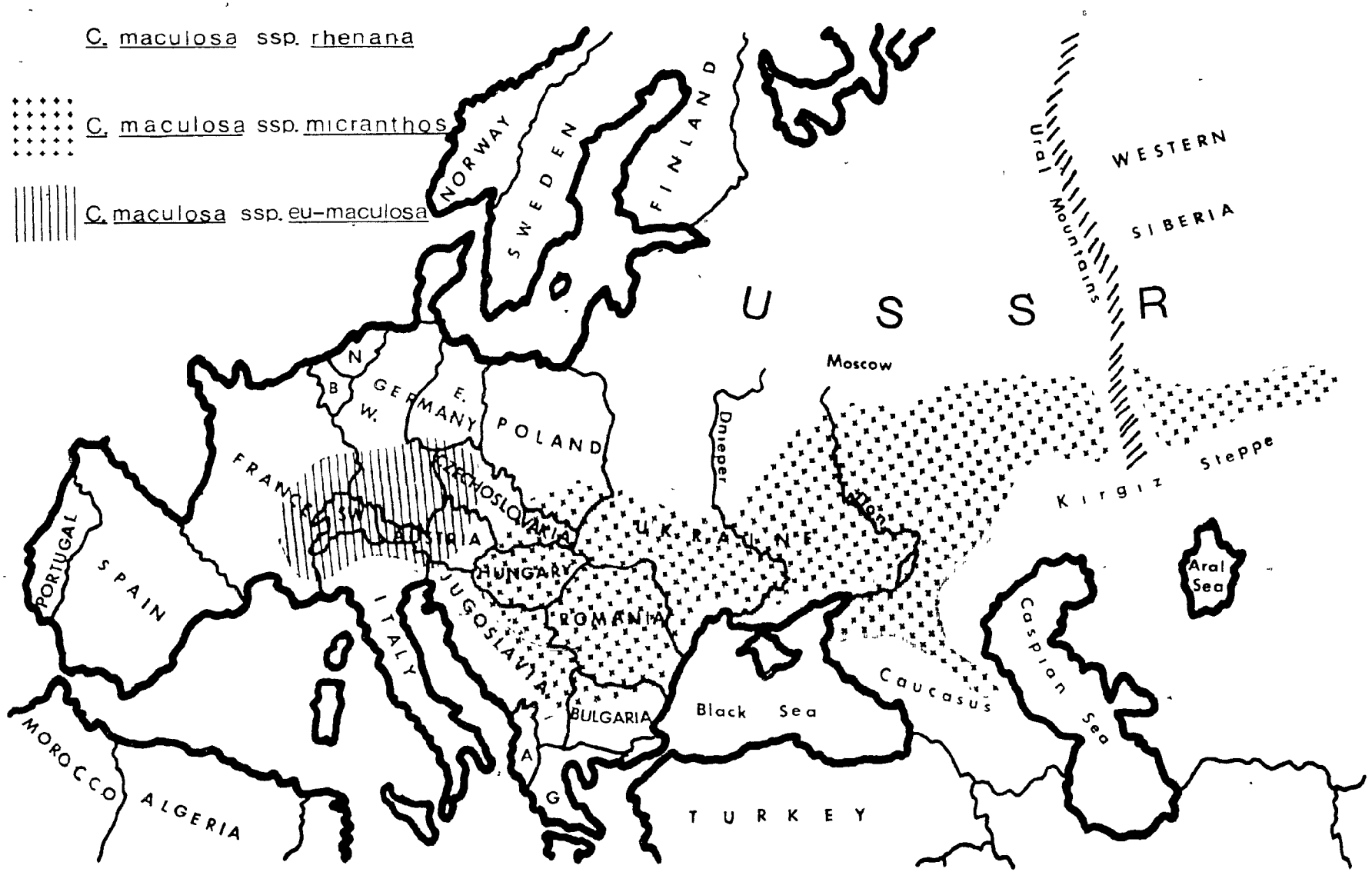
differences exist in North American populations of spotted knapweed.

2.3 Geographical distribution

C. maculosa Lam. is of Eurasian origin and its native distribution comprises central Europe, northward to northern France and Germany, south to the Pyrenees, northern Italy and the northern Balkans, eastward to central Russia, Caucasus, and western Siberia (Moore and Frankton 1974). Figure 1 illustrates the native distribution of the maculosa complex according to Hegi (1912) with ssp. eu-maculosa in southeastern France, northern Switzerland, southern Germany, northern Italy; ssp. rhenana widely distributed throughout central and eastern Europe; and ssp. micranthos in Slovakia, Hungary, Yugoslavia, Romania, western Siberia, and Caucasus.

Although Dostal (1976) uses a different scheme of classification, the following three species have a similar distribution pattern as compared to the three subspecies described above: C. maculosa Lam. with a western distribution (from central France, eastward to southern Germany and northern Italy); C. rhenana is distributed throughout central and southeastern Europe; C. biebersteinii ssp. biebersteinii (=C. micranthos) is found in southeastern Europe and northeastward to north central Ukraine. Therefore, within

Figure 1. Native distribution of the Centaurea maculosa Lam. complex (Hegi 1912).


C. maculosa ssp. rhenana
C. maculosa ssp. micranthos
C. maculosa ssp. eu-maculosa
NORWAY
SWEDEN
FINLAND
U S S R
Ural Mountains
WESTERN SIBERIA
Moscow
N. W. GERMANY
E. GERMANY
POLAND
Dnieper
FRANCE
SWITZERLAND
CZECHOSLOVAKIA
UKRAINE
Kirgiz Steppe
PORTUGAL
SPAIN
HUNGARY
ROMANIA
ITALY
YUGOSLAVIA
BULGARIA
Black Sea
Caucasus
Caspian Sea
Aral Sea
MOROCCO
ALGERIA
TURKEY

the native range of C. maculosa, distinct forms or biotypes do occur and they appear to have characteristic distribution patterns. Whether some or all of these forms should be raised to the species level is debatable. A complete taxonomic evaluation of this species group is necessary to deal satisfactorily with the variation of this weed.

In North America, spotted knapweed is most abundant in the northwestern parts of the continent. In Canada, it is abundant in British Columbia and is common in Ontario, Quebec, Nova Scotia and New Brunswick (Frankton and Mulligan 1970; Moore 1969). Several small infestations have also been reported in southwestern Alberta (Watson and Renney 1974).

In U.S.A., it is found everywhere except possibly the the southeastern states (Moore 1969). It is particularly abundant in Montana, Idaho, Washington, Oregon and northern California (Maddox 1979). The weed is also common in the Great Lakes regions, the lower midwest and the northeastern States (Reed and Hughes 1970).

2.4 Habitat

Spotted knapweed is favored by mesic conditions in the interior of British Columbia where annual precipitations range from 25.1 to 64.8 cm (Watson 1977). Spotted knapweed is

not adapted to the dry conditions of the western prairies. Harris and Cranston (1979) reported that although the dark brown soils of western Canada are susceptible to invasion by spotted knapweed, the weed may be close to its distributional limits because of climatic conditions prevailing in parts of southern Alberta and Saskatchewan.

Spears et al. (1980) have demonstrated that spotted knapweed had better emergence characteristics over a wide range of seeding depth and soil moisture than diffuse knapweed (Centaurea diffusa Lam.). These differences could explain the wider distributional range of spotted knapweed in North America. The present northern limit of spotted knapweed distribution is 55°N latitude in British Columbia (Watson and Renney 1974).

Spotted knapweed does not persist under annual cultivation but invades pastures, rangelands, rights-of-way, roadsides and waste places (Watson 1977). The degree of soil disturbance is a critical factor for its establishment; spotted knapweed is commonly associated with pioneer plant species in the mesic regions of southern British Columbia (Watson and Renney 1974). Spotted knapweed does not compete with vigorously growing grass in moist sites or with diffuse knapweed in steppic grassland (Harris and Cranston 1979).

In Europe, spotted knapweed grows aggressively in the forest steppe regions (Harris and Cranston 1979). It is also a

ruderal species which colonizes disturbed habitats such as quarries and waste places and usually occurs as widely scattered patches (A.K. Watson, pers. comm.). Hegi (1912) has enumerated a list of plant communities with which spotted knapweed is associated in Europe.

2.5 Biology

The biology of spotted knapweed has been reviewed by Watson and Renney (1974). Spotted knapweed is cross-pollinated, but also self-compatible and does not reproduce vegetatively. The plant has a prolific annual seed production (up to 40,000 seeds/m) and overwinters as seeds and/or as a rosette which can regenerate for a few years. Rosettes bolt in early May and flowering occurs in July and August. Seeds are dispersed by wind or machinery. If moisture is adequate, the seeds will germinate and develop into rosettes by fall.

Spotted knapweed has a patchy distribution but large stands are not uncommon in some areas. Populations extend largely through peripheral enlargement of existing stands. The aggressiveness of this weed through its competitive and allelopathic effects on associated species, is reflected in the establishment of single-species stands of spotted knapweed (Fletcher and Renney 1963).

2.6 History

Spotted knapweed was first collected in Canada at Victoria, British Columbia, by Macoun in 1893 (Groh 1944). It is suspected to have been introduced in western North America as a contaminant of alfalfa seed either from Asia Minor or with hybrid alfalfa seed from Germany (Moore 1969). The earliest record in the United States dates from 1894 near a wool waste in Massachussetts and seeds were probably introduced from Central Europe via sheep wool importation (Fletcher 1913)..

Since its introduction, spotted knapweed has spread rapidly and is now estimated to infest approximately 800,000 hectares in Montana and 40,000 ha in both Idaho and Washington (Maddox 1979). In British Columbia, spotted knapweed infests about 4,000 ha and approximately 900,000 ha are threatened by spotted knapweed invasion in western Canada (Harris and Cranston 1979). A few extensive stands are present in Ontario and threaten agricultural lands in some counties (Frankton and Mulligan 1970). In Quebec, spotted knapweed was first collected in 1932 at Iberville and infestations are localized and persistent in the southern regions (Rousseau 1968).

2.7 Costs and Losses

Economic losses from spotted knapweed are the result

of the weed's ability to displace native herbaceous vegetation or superior forage species to the detriment of both ranching and wildlife. In British Columbia, knapweeds (C. maculosa and C. diffusa) were estimated to cause up to 88% forage reductions in infested areas, representing an annual loss of $350,000 (Can. funds) (Harris and Cranston 1979). In western United states, the total annual loss was approximately $600,000 (U.S. funds)in 1979 (Maddox 1979).

Other detrimental attributes of spotted knapweed are its low nutritive value, high fiber content and allelopathic effects on other plant species (Watson and Renney 1974).

2.8 Beneficial

Spotted knapweed provides substantial nectar and pollen for bees, but the nectar has a bitter taste which lowers the quality of the honey (Watson and Renney 1974). An antibacterial substance has been isolated from the leaves and flower heads of spotted knapweed (Cavallito and Bailey 1949; Monya et al. 1968).

Despite the fact that spotted knapweed is a valuable pioneer species capable of reducing erosion and possibly providing food and shelter for birds and rodents, the ecological benefits are limited because this species should

not contribute more than the species it displaced in the natural community. Since knapweed displaces native vegetation and occurs as single-species stands, this reduction of the flora complexity results in an ecological loss (Andres 1981).

2.9 Control methods

Spotted knapweed can be selectively killed in grass with picloram (4-amino-3,5,6-trichloropicolinic acid) at .40-.55 kg/ha but not without disadvantages. Picloram has a residual life in the soil of about 4 years which limits reseeding of grasses and spotted knapweed can reinfest the treated area if further treatments are not applied. Localized patches can be controlled with 2,4-D ester ((2,4-dichlorophenoxy)acetic acid) at 2.2 kg/ha prior to bud formation but follow-up treatments are necessary the next season (Expert Committee on Weeds, Western Canada Section 1983).

Although effective, the cost of chemical control over the 840,000 ha infested with spotted knapweed is prohibitive , especially since the infestations occur primarily on land of low economic value (Harris and Cranston 1979; Maddox 1979).

Spotted knapweed is not a problem in cultivated land

and the plant can be controlled by cultivation and suppressed by mowing. However, the topography of the rangeland commonly infested does not permit the use of farm machinery (Harris and Cranston 1979).

Invasion of spotted knapweed can be slowed down by the seeding of vigourous grass species such as crested wheatgrass (Hubbbard 1970). However, the success of this managerial method of control is still dependent upon chemical treatments and cultural practices which in turn restrict its application in dryland situations (Harris and Cranston 1979).

3.0 Biological control

Since spotted knapweed is difficult to control by chemical, cultural, and managerial methods, biological control may represent an economic and long-term alternative (Harris and Cranston 1979). Watson and Renney (1974) have listed the insects collected on spotted knapweed in North America, but none of these insects inflicted serious damage on the plant. Also, no microorganisms have been reported to attack spotted knapweed in the field.

A survey of phytophagous insects attacking wild Cynarae (Asteraceae) was initiated in Europe in 1961 (Zwölfer 1965). Schroeder (1977) has published a list of biotic agents attacking knapweeds in Europe with their prospective

suitability for biological control. Sixteen species of insects, one species of mite, and two species of rust pathogens were suggested to be possible biocontrol agents. To date, four species of insects have been introduced and released in North America for the biological control of spotted knapweed (Table 1).

Although the Urophora seed-head flies have reduced seed production of spotted knapweed by as much as 75% at release sites in British Columbia, this level of attack has not reduced the population of this weed (Harris and Cranston 1979). Both species of Urophora have made an important contribution toward reducing the aggressiveness of spotted knapweed, but it is generally agreed that additional agents will be required to achieve satisfactory biological control (Harris 1980a; Maddox 1982).

Table 1. List of insects released in North America for the biological control of spotted knapweed (Centaurea maculosa Lam.)

Agent	Date first Released	Origin	Status	References
Agapeta zoegana L. (Lepidoptera: Cochylidae)	1982	Austria, Romania, Hungary	Not established in British Columbia but attempts continuing	Muller et al. 1982
Metzenaria paucipunctella Zeller (Lepidoptera: Gelechiidae)	1973	Switzerland	Increased to attack ⅓-½ heads at B.C. release site but suffering high winter mortality and destroy U. affinis in same head.	Harris and Myers 1984 Meyers and Ockenden 1977.
Urophora affinis Frauenfeld (Diptera: Tephritidae)	1970	France	Forms 3.3 to 5.0 galls per head in combination with U. quadrifasciata in B.C. release sites.	Harris 1980 a,b Maddox 1982
	1973	France	Forms up to 2.25 galls per head in Montana and an average of 1.61 galls per head in Idaho. Established in Quebec	Storey and Anderson 1978 Watson 1983 (pers. comm.)
Urophora quadrifasciata (Meigen) (Diptera: Tephritidae)	1972	U.S.S.R.	In B.C., partially displaced by U. affinis. Established in Quebec.	Harris 1980 a,b Watson 1983. (pers. comm.)

CHAPTER III. RUST FUNGI ATTACKING CENTAUREA MACULOSA LAM.

Four species of autoecious rusts have been reported to attack Centaurea maculosa Lam. in Europe; Puccinia centaureae DC; P. centaureae-vallesiacae Hasler (a variant of P. centaureae); P. jaceae Otth; and P. verruca Thuemen (Gaumann 1959; Guyot 1967). Their taxonomy is as difficult and confused as the taxonomy of their host genus Centaurea.

3.1 Puccinia centaureae DC.

3.1.1 Taxonomy

In 1815, A.P. de Candolle described this rust collected on Centaurea scabiosa L. (Guyot 1967). Like most of the species created by de Candolle, P. centaureae was insufficiently characterized initially, which consequently led to different interpretations of this species by taxonomists.

P. centaureae was first examined morphologically and experimentally by Jacky (1899). He distinguished two morphological forms or types which differ mainly by the number and position of urediniospore germpores: Type A with two

super-equatorial germpores and Type B with three equatorial germpores . He also found, in connection with the biological behavior of this rust species, that two formae speciales are distinguishable with one on Centaurea jacea L. and the other on Centaurea nervosa Willd..

Other variants of P. centaureae have subsequently been described (Gaumann 1959; Guyot 1967; Savile 1970a, b). These variants represent biological forms of the rust species which are well adapted to their particular host and can be identified by their morphological characteristics and by comparing their respective host ranges. The taxonomic position of these variants is however not clear and the exact disposition of some of these variants would require a longer series of specimens than is now available and good host vouchers (Savile 1970b). Although the host range of some P. centaureae variants has been determined experimentally, only a few Centaurea species were tested. Nevertheless, a level of host specialization has been demonstrated for certain variants of P. centaureae (Gaumann 1959; Ialongo and Boldt 1977; Jacky 1899).

The only evidence of a variant that infects C. maculosa comes from Hasler (1908) who experimentally determined the host range of a rust collection from Centaurea vallesiaca (DC.)Jordan in Switzerland. In addition to the original host, this rust would infect Centaurea alba L., C. axillaris Willd., C. cyanus L., C. maculosa Lam., C. rhenana

Bor., but was unable to infect C. austriaca Willd., C. jacea L., C. nervosa Willd., C. nigra L., C. nigrescens Willd., C. phrygia L., C. scabiosa L. and C. transalpina Schleider ex DC. He named this rust Puccinia centaureae-vallesiacae Hasler.

In his review of the rust fungi on Compositae in North America, Cummins (1978) included P. centaureae, P. carthami Cda., P. cirsii Lasch in Rabh., P. laschii Lagerh., and P. irrequisita H.S. Jack. as synonyms of Puccinia calcitrapae var. centaureae (DC.) Cumm. Wilson and Henderson (1966) have previously adopted a similar broad classification in Britain, including P. centaureae and other related species under P. calcitrapa DC. Savile (1970a) disagrees with such an ultra-broad species concept and stated "including all these taxa under Puccinia calcitrapae, a rust seemingly confined to Centaurea calcitrapa, effectively suppresses all the biological information made available by a more precise and realistic treatment". Savile (1970b) recognizes four full species on North American Cirsium species including many host limited varieties. Less detailed studies have demonstrated appreciable diversification of European rusts attacking Carduus , Carthamus , Centaurea and other related genera (Savile 1970a).

Morphological and biological features of P. centaureae sensu stricto are listed in Table 2. Studies on urediniospore morphology have indicated distinct differences

Table 2. Comparative biological and morphological features of Puccinia centaureae DC., P. jaceae Otth. and P. verruca Thum.

		Puccinia centaureae [a,b]	Puccinia jaceae [a,b]	Puccinia verruca [a,b,c]
LIFE CYCLE		MACROCYCLIC, AUTOECIOUS 0 (RARELY SEEN), II, III	MACROCYCLIC, AUTOECIOUS 0 (RARELY SEEN), II, III	MICROCYCLIC, AUTOECIOUS III
UREDINIOSPORE	SIZE	21-28(19-31) X 18-25(16-28)	21-31(20-34) X 19-29(18-33)	
	$\overline{X}$	22-26 X 20-23	23-30 X 21-26	
	SHAPE	SPHERICAL AND SYMMETRICAL	BROADLY ELLIPSOID AND FLATTENED	
	GERMPORE	3(RARELY 2,4) EQUATORIAL	2 SUPEREQUATORIAL	
	HILUM	MINUTELY VERRUCOSE	SMOOTH	
TELIOSPORE	SIZE	26-40(24-45) X 17-28(16-30)	28-42(24-46)(→ 48) X 20-28(16-32)	28-60(32-72) X 13-24(11-28)
	$\overline{X}$	30-36 X 18-26	31-38 X 21-26	42-50 X 15-18
HOST RANGE (SEE TABLE 3)		162 CENTAUREA SP.	43 CENTAUREA SP.	14 CENTAUREA SP. AND 2 CARTHAMUS SP.

[a] Guyot 1967

[b] Savile 1970 a, b

[c] Sydow and Sydow 1904

0 Pycnia

II Uredinia

III Telia

in spore ornamentation between P. centaureae and P. carthami (Savile 1970a, b; Traquair and Kokko 1983). The safflower rust, P. carthami, can be distinguished from P. centaureae by its more verrucose hilum and shorter echinulae. Furthermore, P. carthami has never been reported on any Centaurea species in field conditions (Arthur 1962; Conners 1943), but was reported on C. cyanus inoculated in a greenhouse (Savile 1944).

3.1.2 Host records

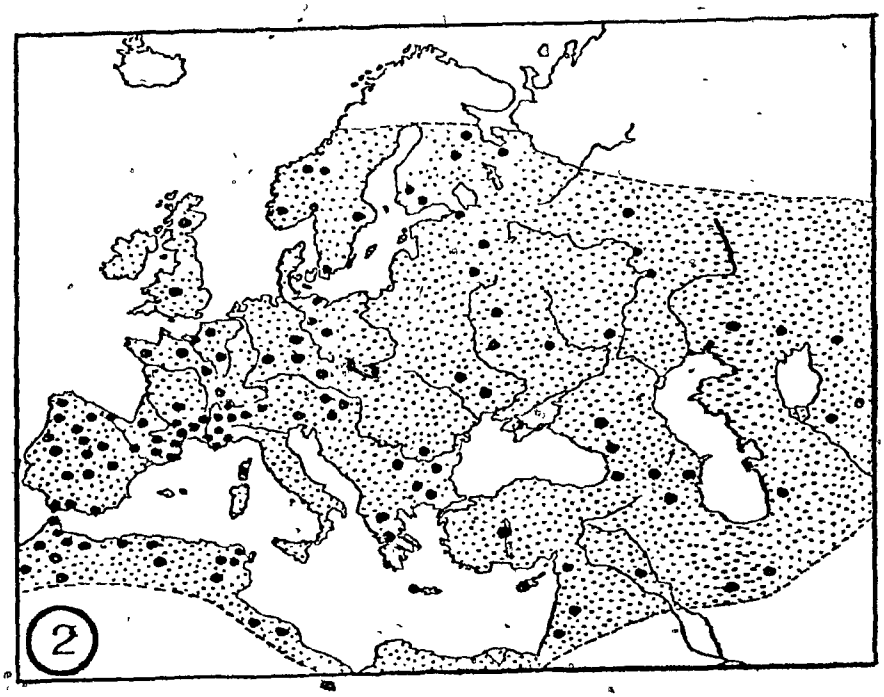
P. centaureae has been recorded on more than 162 species of Centaurea (Table 3). Despite this broad host range, a number of host-limited varieties has been reported in the literature (Ialongo and Boldt 1977; Jacky 1899; Gaumann 1959; Guyot 1967; Savile 1970a, b; Savile 1973). This rust species has never been reported to attack plants in genera other than Centaurea.

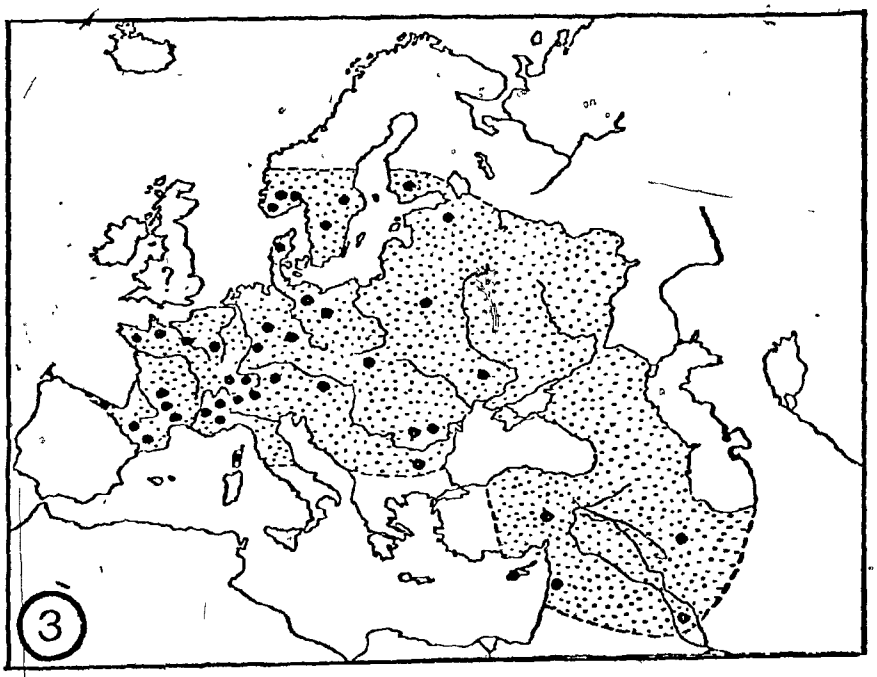
3.1.3 Geographical distribution

The geographical distribution of P. centaureae sensu stricto comprises most of the European continent, northern Africa and central Asia, with the exception of the Arctic Highlands, Siberia, China and Japan (Figure 2).

Figure 2. Geographic distribution of Puccinia centaureae DC. sensu stricto. (Guyot 1967)

Figure 3. Geographic distribution of Puccinia jaceae Otth. (Guyot 1967).


2


3

This rust species was not known to occur in North America until 1965 when Savile collected it on Centaurea nigra L. at Indian Point, Nova Scotia. He identified the specimen as Puccinia centaureae DC. var. centaureae and considered it as a biotype of the same rust found in Europe (Savile 1970b). This rust was later found to be weakly virulent on spotted knapweed in host range studies (A.K. Watson, pers. comm.).

3.1.4 Habitat

P. centaureae is mainly distributed in the mesic regions of Europe and Asia where it is most often found in the plains, and also in plateau regions reaching 1,400 metres in altitude and also on mountain slopes up to 1,700 metres (Guyot 1967). This rust shows climatic adaptations in urediniospores, teliospores, or both against the extremely dry summers of the Mediterranean regions (Savile 1970a). P. centaureae distribution encompasses the full native range of the spotted knapweed complex.

3.2 Puccinia jaceae Otth

3.2.1 Taxonomy

This species was described by G. Otth in 1865 from Centaurea jacea L. in Switzerland (Guyot 1967). Jacky (1899),

who probably examined the specimen, stated that it corresponded to his Type A of P. centaureae because of the presence of two super-equatorial urediniospore germpores. Sydow and Sydow (1904) agreed with Jacky and reported P. jaceae as a synonym of P. centaureae in their Monographia Uredineanum.

The views of other taxonomists are divergent. Some authors recognize the validity of these two morphological characters, number and position of urediniospore germpores, for maintaining the autonomy of both species (Gaumann 1959; Hasler 1908). Others consider, by referring to the Sydows, that these differences in spore morphology are insufficient to Trotter 1908).

More recently, Savile (1970a,b) has treated the two species under separate evolutionary lineages: the Puccinia dioicae-P. hieracii complex (which includes P. jaceae) and the Puccinia centaureae-P. laschii complex (which includes P. carthami). Recent studies on urediniospore morphology of P. centaureae, P. jaceae and P. carthami seem to support Savile's classification (Traquair and Kokko 1983).

The main morphological and biological features of P. jaceae are listed in Table 2. P. jaceae can be distinguished from P. centaureae by having larger, broadly ellipsoidal, and flattened urediniospores with two super-equatorial germpores and a smooth hilum. P. centaureae has smaller, spherical,

and symmetrical urediniospores with three equatorial germpores and a minutely and distinctly verrucose hilum.

3.2.2 Host records

Many Centaurea species have been reported to harbor this rust species in Europe but fewer hosts are reported for P. jaceae than P. centaureae (Table 3). Common hosts for both rust species are not rare and mixed infections on the same host have been reported (Watson et al 1981).

Savile (1970a) has described three varieties of P. jaceae in Europe. Watson and Alkhoury (1981) have experimentally determined the host range of a variant collected on Centaurea diffusa in Romania and reported a high level of pathogenicity on C. diffusa Lam., C. cyanus L., C. nigra L. and Carthamus tinctorius. Two Centaurea species, C. maculosa and C. montana L., were resistant to the rust isolate.

3.2.3 Geographical distribution

P. jaceae has a more restricted distribution than P. centaureae (Figure 3). It occupies much of the European continent, with the exception of the British Isles, and does

not occur as far south as P. centaureae. The eastern limits of the P. jaceae range are Asia Minor and the Near East. This species has not been reported in North America (Savile 1970b).

3.3 Puccinia verruca Thumen

3.3.1 Taxonomy

This rust was described by F. von Thumen in 1879 on Centaurea napifolia L. in Upper Egypt (Guyot 1967). It is a microcyclic rust where only the telial stage is present. The telia are grouped together giving a typical verruciform pustule 1 to 4 mm in diameter on the surface of the leaf. The teliospores are narrow, smooth, usually thickened at the apex and with a long persistent pedicel (Sydow and Sydow 1909). The dimensions and forms of teliospores vary greatly even within a sorus (Table 2).

3.3.2 Host records and geographical distribution

This rust has been collected on fourteen Centaurea species in Europe, Asia and northern Africa (Table 3). It was reported on Carthamus tinctorius in Russia and on C. lanatus L. in France and Tunisia (Guyot 1967). The existence of this

rust on Centaurea maculosa is questioned. Sydow and Sydow (1904) reported it on C. maculosa but after careful examination of their specimen, Guyot (1967) found no trace of teliospores and attributed the presence of warts on leaf surface to an entomophagous origin.

Table 3. Comparative host records of Puccinia centaureae DC. sensu stricto, P. jaceae Otth and P. verruca Thum. (Guyot 1967).

Host Species	P. centaureae	P. jaceae	P. verruca
Centaurea acaulis Desf.	+	-	-
C. achaia B. et H.	+	-	-
C. adami W.	+	-	-
C. alba L.	+	+	-
C. alexandri Bordz	+	-	-
C. ali-beyana F.Q. et Pau.	+	-	-
C. alpestris H. et H.	+	-	-
C. alpina L.	+	-	-
C. amara L.	+	+	-
C. americana Nutt.	+	-	-
C. angustifolia Schrank	+	+	-
C. arenaria M.B.	+	-	-
C. aspera L.	+	-	-
C. atrata W.	+	-	-
C. atropurpurea W. et K.	+	-	-
C. aurantiaca Willd.	+	-	-
C. austriaca Willd.	+	+	-
C. axillaris Willd.	+	-	-
C. badensis Tratt.	+	-	-
C. balansae Boiss. et Reut.	+	-	-
C. balsamita Lam.	+	-	-
C. banatiea Roch.	+	-	-
C. beckeriana Wagn.	+	-	-
C. behen L.	+	+	-
C. belangeri DC.	+	-	-
C. bella Trautv.	+	+	-
C. beltranii Pau.	+	-	-
C. benoistii Humb.	+	-	-
C. beskideana W. et M.	+	-	-
C. bracteata Scop.	+	+	-
C. breviceps Hjin.	+	-	-
C. brevispina Hansskn.	+	-	-
C. calcarea Jord.	+	-	-
C. calcitrapa L.	+	+	-
C. cana Sm.	+	-	-
C. canariensis Willd.	+	+	-
C. carduiformis DC.	+	-	-
C. cariensis Boiss.	+	-	-
C. carpetana Boiss. et Reut.	+	-	-
C. castellana B. et R.	+	-	-

+ Host species on which the rust has been recorded.

Table 3. (Continued)

Host Species	P. centaureae	P. jaceae	P. verruca
C. cetia (Beck) Wagner	+	-	-
C. cheiracantha Fenzl.	+	-	-
C. cheirolopha (Fenzl.) Wagenitz	+	-	-
C. chrysolepis Vis.	+	-	-
C. cirrahata Rchb.	+	+	-
C. collina L.	+	-	-
C. conglomerata C.A. Mey.	+	+	-
C. contracta Viv.	+	-	-
C. coriacea W. K.	+	-	-
C. cyanoides B. et W.	+	-	-
C. cyanus L.	+	+	-
C. cyrtolepis Led.	+	-	-
C. dealbata W.	+	+	-
C. decipiens Thuill.	+	-	-
C. diffusa Lam.	+	+	-
C. diluta Ait.	+	-	-
C. dimorpha Viv.	+	-	-
C. dubia Suter	+	+	-
C. endressii Hochst. et Steud.	+	+	-
C. ensiformis P. II.	+	-	-
C. eriophora L.	+	-	-
C. eryngioides Lam.	+	-	-
C. exarata Boiss.	+	+	-
C. fenzlii Reich.	+	+	-
C. flavida Nyar.	+	+	-
C. fragilis D. R.	+	-	-
C. gentilii Br. Bl. et Maire	+	-	-
C. glastifolia L.	+	+	-
C. glomerata Vahl	+	-	-
C. guicciardii Boiss.	+	-	-
C. hanryi Jord.	+	-	-
C. homeosceros Pau.	+	-	-
C. hyalolepis Boiss.	+	+	-
C. iberiea Trev.	+	+	-
C. idaea B. et H.	+	-	-
C. indurata Janka	+	-	-
C. infestans Coss. et Dur.	+	-	-
C. involucrata Desf.	+	-	-
C. jacea L.	+	+	+
C. jungens Gugl.	+	+	-
C. kermanensis Bornm.	+	-	-
C. kotschyana Heuff.	+	-	+
C. kroumirensis Cosson	+	-	-
C. linaresii Laz.	+	-	-
C. litardierei Jah. et Maire	+	-	-
C. lydia Boiss.	+	-	-

Table 3. (Continued)

Host Species	P. centaureae	P. jaceae	P. verruca
C. macedonica Griseb.	+	-	-
C. macrocephala Muss. Push.	+	-	-
C. maculosa Lam.	+	+	+
C. margaritacea Ten.	+	-	-
C. maroccana Ball.	+	-	-
C. melitensis L.	+	-	-
C. meryonis DC.	+	-	-
C. micranthos Gmel.	+	-	-
C. minoa Heldr.	+	-	-
C. montana L.	+	-	+
C. monanthos Georgi	+	-	-
C. muntgoi	+	-	-
C. mureti Jord.	+	-	-
C. musimomum Maire	+	-	-
C. myriocephala Sch. et Bip.	+	+	-
C. nana Desf.	+	-	-
C. napifolia L.	+	-	+
C. nervosa Willd.	+	+	-
C. nicaeensis All.	+	-	+
C. nicolai Baldacci	+	-	-
C. nigra L.	+	+	-
C. nigrescens Willd.	+	+	-
C. orientalis L.	+	-	+
C. ornata Willd.	+	-	-
C. ossica C. Koch	+	-	-
C. ovina Pall.	+	-	-
C. oxylepis Wim. et Grab.	+	-	-
C. paniculata L.	+	+	-
C. pannonica Heuff.	+	+	-
C. phrygia L.	+	+	+
C. phyllocephala Boiss.	+	-	-
C. pleeskensis Nyar.	+	+	-
C. plumosa Kern.	+	-	-
C. polyacantha	+	-	-
C. polypodifolia DC.	+	-	-
C. pratensis Thuill.	+	-	-
C. procurrens Sieb.	+	-	-
C. pseudophrygia C.A. Mey.	+	-	-
C. pubescens Willd.	+	-	-
C. pugioniformis Nyar.	+	+	-
C. pulchella Ledeb.	+	-	-
C. pullata L.	+	-	+
C. recta Krock.	+	-	-
C. rhapontieum Will.	+	-	-
C. rhenana Bor.	+	-	-
C. rivularis Brot.	+	-	-
C. ruthenica Lam.	+	+	-
C. romana L.	-	-	+

Table 3. (Continued)

Host Species	P. centaureae	P. jaceae	P. verruca
C. sadleriana Janka	+	-	-
C. salicifolia M. B.	+	+	-
C. salonitana Vis.	+	-	-
C. scabiosa L.	+	-	+
C. schizolepis Trautv.	+	-	-
C. seridis L.	+	-	-
C. sessilis Willd.	+	-	-
C. solstitialis L.	+	+	-
C. sonchifolia L.	-	-	+
C. sphaerocephala L.	+	-	-
C. spinulifolia L.	-	-	+
C. spinulosa Roch.	+	-	+
C. splendens Tenore	+	-	-
C. spruneri B. et H.	+	-	-
C. squarrosa Willd.	+	+	-
C. stenolepis A. Kern.	+	+	-
C. stereophylla Bess.	+	-	-
C. sterilis Stev.	+	-	-
C. stoebe L.	-	+	-
C. sub-fleischeri Nyar.	+	+	-
C. sulphurea Willd.	+	-	-
C. szollosii Wagner	+	-	-
C. szovitsiana Boiss.	+	-	-
C. tauscheri Kern.	+	-	-
C. transalpina Schleich.	+	+	-
C. transcaucasica P. Sosn.	+	-	-
C. trichocephala M.B.	+	-	-
C. triumfetti All.	+	-	-
C. uniflora L.	+	-	-
C. vallesiaca Jord.	+	-	-
C. vesceritensis Boiss. et Reut.	+	-	-
C. virgata L.	+	+	-
Carthamus tinctorius L.	-	-	+
C. lanatus L.	-	-	+

EXPERIMENTATION

CHAPTER IV. SURVEY FOR AND COLLECTION OF RUST FUNGI ON *CENTAUREA MACULOSA* LAM. IN EASTERN AND CENTRAL EUROPE.

4.1 Introduction

An evaluation of the available information on the biology and control of spotted knapweed in North America initiated the search for additional biocontrol agents in the native range of this noxious weed. At present, the chemical, cultural, and managerial methods of control have proven to be ineffective and/or unfeasible for the thousands of hectares infested with spotted knapweed in western North America. The four insects that were imported from Europe and released in North America for the biological control of spotted knapweed have not contributed significantly in reducing the aggressiveness of this weed . The search for additional biocontrol agents could result in finding other organisms that would, alone and/or in combination with those biocontrol agents already established, contribute to significant suppression of this noxious weed.

There is evidence in the literature that rust pathogens attacking *C. maculosa* in Europe would be suitable

for investigation as possible biocontrol agents of spotted knapweed (Guyot 1967; Savile 1970 a,b). Rust fungi have proven in the past to be effective biocontrol agents against weeds, as demonstrated with Puccinia chondrillina Bubak & Syd. against skeletonweed (Chondrilla juncea L.) in Australia (Hasan 1972); Puccinia xanthii against Xanthium species. (Hasan 1974b); and blackberry rust, Phragmidium violaceum (Schulz) Winter, against weedy Rubus species in Argentina (Oehrens 1977). These rusts are autoecious and have demonstrated a very high level of specialization on their hosts. Autoecious rust fungi are more suitable for use in biological control of weeds than heteroecious rust fungi, since in the later case the alternate host may be a useful plant or may be absent from the target area. Also, experimentation with heteroecious rusts is difficult to conduct. While working with rumex rust, Uromyces rumicii (Schum.)Win., for the biological control of curly dock (Rumex crispus L.), Inman (1971) could not succeed in infecting the alternate host Ranunculus ficaria L.. Puccinia species attacking spotted knapweed in Europe are autoecious, macrocyclic rusts with host ranges limited to a single host or to a group of Centaurea sp. (Gaumann 1959; Guyot 1967; Ialongo and Boldt 1977; Savile 1970a: Watson and Alkhoury 1977). These rust pathogens are strong prospects for the biological control of spotted knapweed, and are widespread in Europe encompassing the full native range of spotted knapweed where their search and collection should be concentrated.

Efficient search and sampling strategies for natural enemies of introduced weed species has been described by Marshal et al. (1981) and Wapshere (1981a). It is generally agreed that the center of evolution, or diversification, of the genus and sub-genus of the target weed should be the first priority for exploration (Goeden 1974; Room 1980; Wapshere 1975). This reasoning is based on the assumption that these centers will be the richest source of organisms that have co-evolved with their host plants (Harris 1971). This approach has been confirmed for Ambrosia species in the Sonoran Desert (Harris and Piper 1970), Solanum species in northern Mexico (Goeden 1971), Chondrilla species in southern U.S.S.R. (Wapshere 1974a) and Echium species on the Iberian Peninsula (Wapshere 1981b). The long process of co-evolution of the pathogen with its host plant has resulted, according to many authors, in the accumulation of distinct types and level of protection in the host population and a broad spectrum of virulence in the pathogen (Browning 1981; Leppik 1970; Nelson 1979; Zhukovsky 1959). The initial exploration for suitable agents should be centered at and radiate from such centers of diversification. The search area should be large enough to encompass a large diverse natural enemy complex especially if different weed forms and biotypes of the agent exist (Sands and Harley 1981).

There has been emphasis on collecton of agents from ecoclimatic situations similar to those occupied by the target

weed (Wapshere 1975). Although agents selected from such regions may be more likely to become established and be effective [for example, Puccinia chondrillina on skeltonweed (Wapshere 1978)], many other factors, such as the relative competitiveness of the weed and other components, can influence the effectiveness of an agent (Sands and Harley 1981; Winder and Harley 1978).

In cases where the center of generic diversification cannot be determined for the weed and an ecoclimatic analogous region in the native range cannot be found, it is recommended that a random search be initiated throughout the native range in order to collect a wide genetic stock of the agents (Wapshere 1981a).

The first collections of rust fungi attacking spotted knapweed in Europe were made in 1980 by researchers of the Commonwealth Institute of Biological Control (C.I.B.C.), in Délemont, Switzerland. Five rust samples collected on spotted knapweed in Austria and one sample collected in France were sent to the quarantine facility of Macdonald College. These six collections were found to be of low virulence on eight North American populations of spotted knapweed. Consequently, a more extensive survey was conducted in Eastern and Central Europe to find more virulent strains of the rusts.

4.2 Materials and Methods

The field survey and collection trip was made during the last two weeks of June 1982. Most of the sites were located during previous explorations by entomologists of the Commonwealth Institute of Biological Control, stationed in Delemont, Switzerland. The geographic regions surveyed included western Romania, western Hungary, southeastern Austria, and the Rhine valley near the border of France and Germany.

The sites were selected simply on the basis of a large spotted knapweed population and they were all located within the native range of this species. This strategy has previously resulted in the discovery of virulent and host-specific strains of Puccinia chondrillina Bubak & Syd. on skeletonweed in southern Europe (Hasan 1972, 1981). The size of the spotted knapweed infestation was estimated at each location and corresponded to the area of the collection site. The degree of coverage and sociability of spotted knapweed was also estimated visually at each site using the Braun-Blanquet classification (1932). Observations on the incidence and severity of rust disease in spotted knapweed populations were also made.

Each collection consisted of leaves with rust pustules taken from a single infected plant chosen at random. The leaf sample was rated for reaction to the rust, using the scale of 0 to 4 developed by Stakman et al. (1962), and then

placed in a paper envelope. The collections were coded using a system of five digits e.g., HG-01-c, with the first two capital letters identifying the country of origin, followed by two numbers representing the site and a letter was assigned to each plant on which the collection was made. The material was sent or brought back, with official import permits, to the quarantine facility of Macdonald College of McGill University.

4.3 Results and Discussion

4.3.1 Field survey

A total of 30 sites was surveyed in Eastern and Central Europe (Table 4). The field survey was mainly concentrated in the Steinfeld region of southeastern Austria where 18 sites were surveyed (Figure 4). The remaining sites were distributed as follows: 2 sites in northwestern Romania (Figure 5), 4 sites in eastern Hungary (Figure 4), and 5 sites in the Rhine Valley between France and Germany (Figure 6).

Spotted knapweed plants were readily identified in the field by external morphological characters (Figure 8). Identification at the subspecies level was not possible in the field because plants were only at the seedling or bolting stage at the time of the survey. Classification of the three subspecies of Centaurea maculosa is based primarily on morphological features of the flower head (Hegi 1909).

Table 4. Weed and Rust Population Parameters of European Collection Sites.

Site	Locality	Approximated area of site (m^2)	Estimated cover of spotted knapweed[a]	Estimated sociability of spotted knapweed[b]	No. of rust collections	Rating of rust collections[c]
FC-01	Chalampe, France	10	1	1	3	2
FC-02	Blodelstein, France	4	r	1	2	1
FC-03	Roggenhouse, France	18	r	1	3	1
FC-04	Reguisheim, France	100	r	1	2	1
FC-05	Ottmarsheim, France	100	r	1	1	2
GR-01	Istein, Germany	30	1	1	3	2
HG-01	Sopron, Hungary	1,200	r	1	5	1-3
HG-02	Sopron, Hungary	4,000	r	1	1	2
HG-03	Balf, Hungary	900	r	1	1	2
HG-04	Balf, Hungary	10,000	r	1	*	*
OS-01	Hornstein, Austria	40,000	r	3	10	3
OS-02	Mitterndorf, Austria	10,000	1	2	8	3
OS-03	Durnstein, Austria	10,000	r	1	6	2

* No rusted plant found at this site.

Table 4. (Continued)

Site	Locality	Approximated area of site (m^2)	Estimated cover of spotted knapweed[a]	Estimated sociability of spotted knapweed[b]	No. of rust collections	Rating of rust collections[c]
OS-04	Theresenca Feld, Austria	2,500	r	1	3	2
OS-05	Sollenau, Austria	20,000	1	3	8	2
OS-06	Sollenau, Austria	2,500	1	3	3	2-3
OS-07	Eggendoff, Austria	10,000	r	1	7	2-4
OS-08	Neufeld, Austria	5,000	1	1	1	1
OS-09	St-Margarethen, Austria	1,000	r	1	3	3-4
OS-10	Oggau, Austria	5,000	r	1	4	2
OS-11	Donnerskirchen, Austria	20,000	r	1	3	2
OS-12	Sollenau, Austria	2,500	r	1	4	2
OS-13	Sollenau, Austria	2,500	r	1	1	2
OS-14	Sollenau, Austria	250	2	4	6	3-4
OS-15	Neuribohr, Austria	1,000	3	4	5	3
OS-16	Oeynhausen, Austria	100	r	1	3	3

Table 4. (Continued)

Site	Locality	Approximated area of site (m^2)	Estimated cover of spotted knapweed[a]	Estimated sociability of spotted knapweed[b]	No. of rust collections	Rating of rust collections[c]
OS-17	Richardhorf, Austria	6	r	1	6	3-4
OS-18	Vosendorf, Austria	5	5	1	1	4
RM-04	Crisulic, Romania	100	r	1	1	3
RM-05	Poieni, Romania	100	r	1	1	3

[a] Braun-Blanquet classification of plant cover

Class	Degree of cover
5	76-100% of the area
4	51- 75% of the area
3	26- 50% of the area
2	6- 25% of the area
1	1- 5% of the area
+	Less than 1% of the area
r	Extremely small portion of the area; usually only one specimen

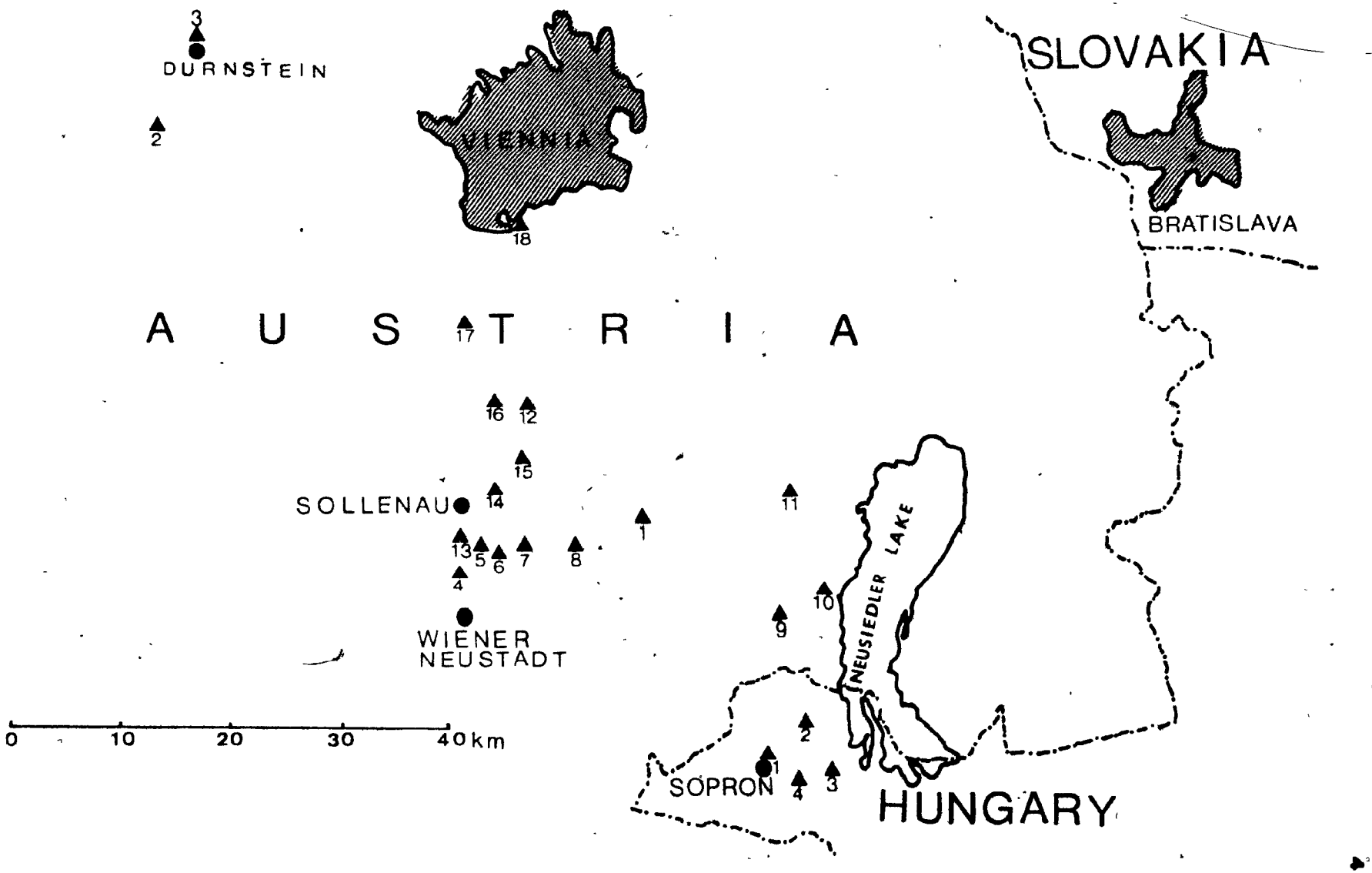
[b] Braun-Blanquet sociability scale

Class	Sociability
1	Growing singly
2	Growing in tufts
3	Growing in small groups
4	Growing in larger groups
5	Growing in extensive groups

[c] Rust rating system

Host reactions	
0	Immune, no symptoms
0;	Nearly immune, hypersensitive spots, no uredinia.
1	Very resistant, minute uredinia surrounded by necrotic area.
2	Moderately resistant, small to medium sized uredinia, chlorosis.
3	Moderately susceptible, medium sized uredinia, no necrosis, may be some chlorosis.
4	Very susceptible, large uredinia often coalescing, no necrosis, may be some chlorosis.

Figure 4. Collection sites (▲) of rust fungi on C. maculosa Lam. in Austria and Hungary.


3
DURNSTEIN
2
VIENNA
18
SLOVAKIA
BRATISLAVA
A U S T R I A
17
16
12
15
SOLLENAU
14
13
5
6
7
8
4
1
11
10
9
NEUSIEDLER LAKE
WIENER
NEUSTADT
0
10
20
30
40km
2
1
4
3
SOPRON
HUNGARY

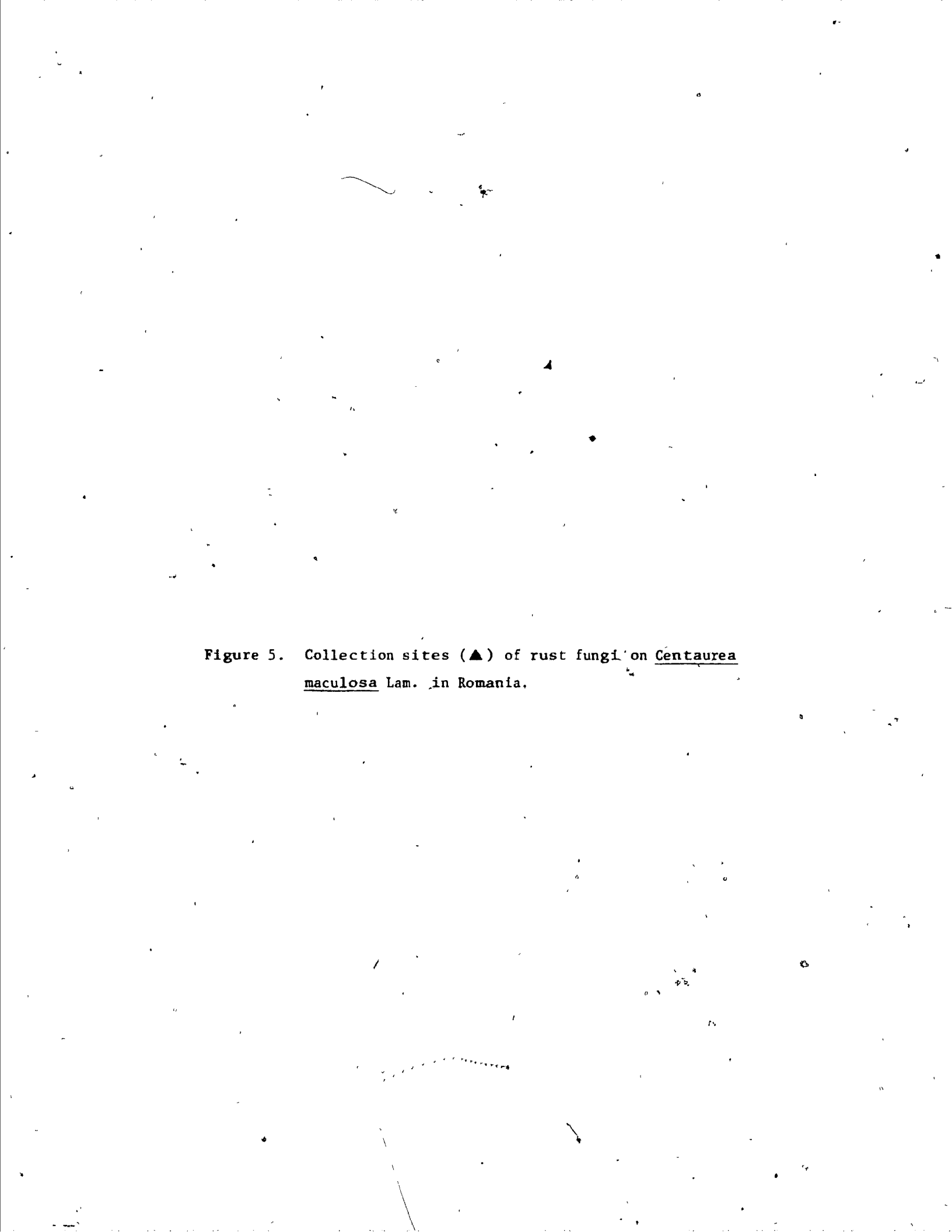


Figure 5. Collection sites (▲) of rust fungi on Centaurea maculosa Lam. in Romania.

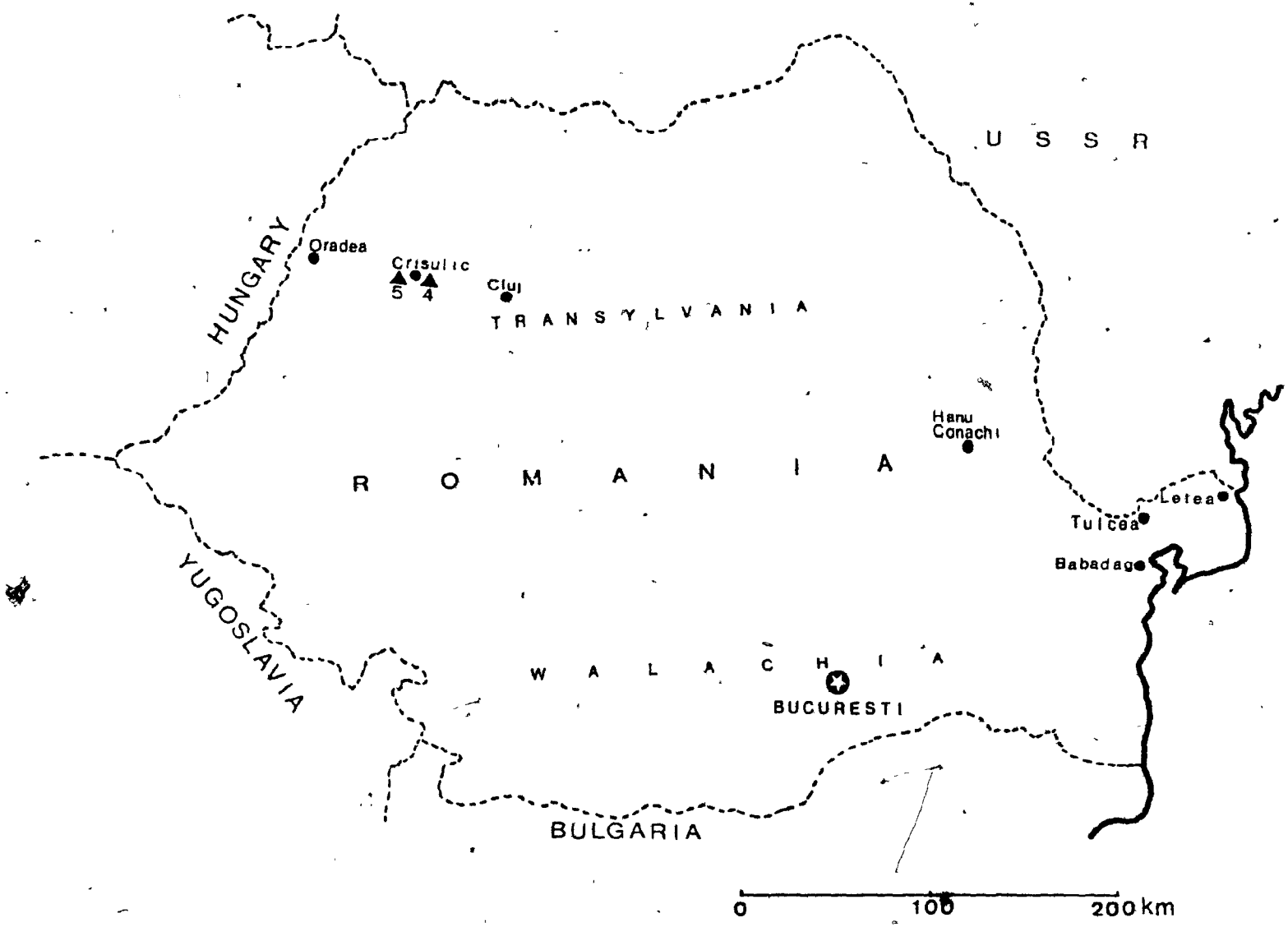
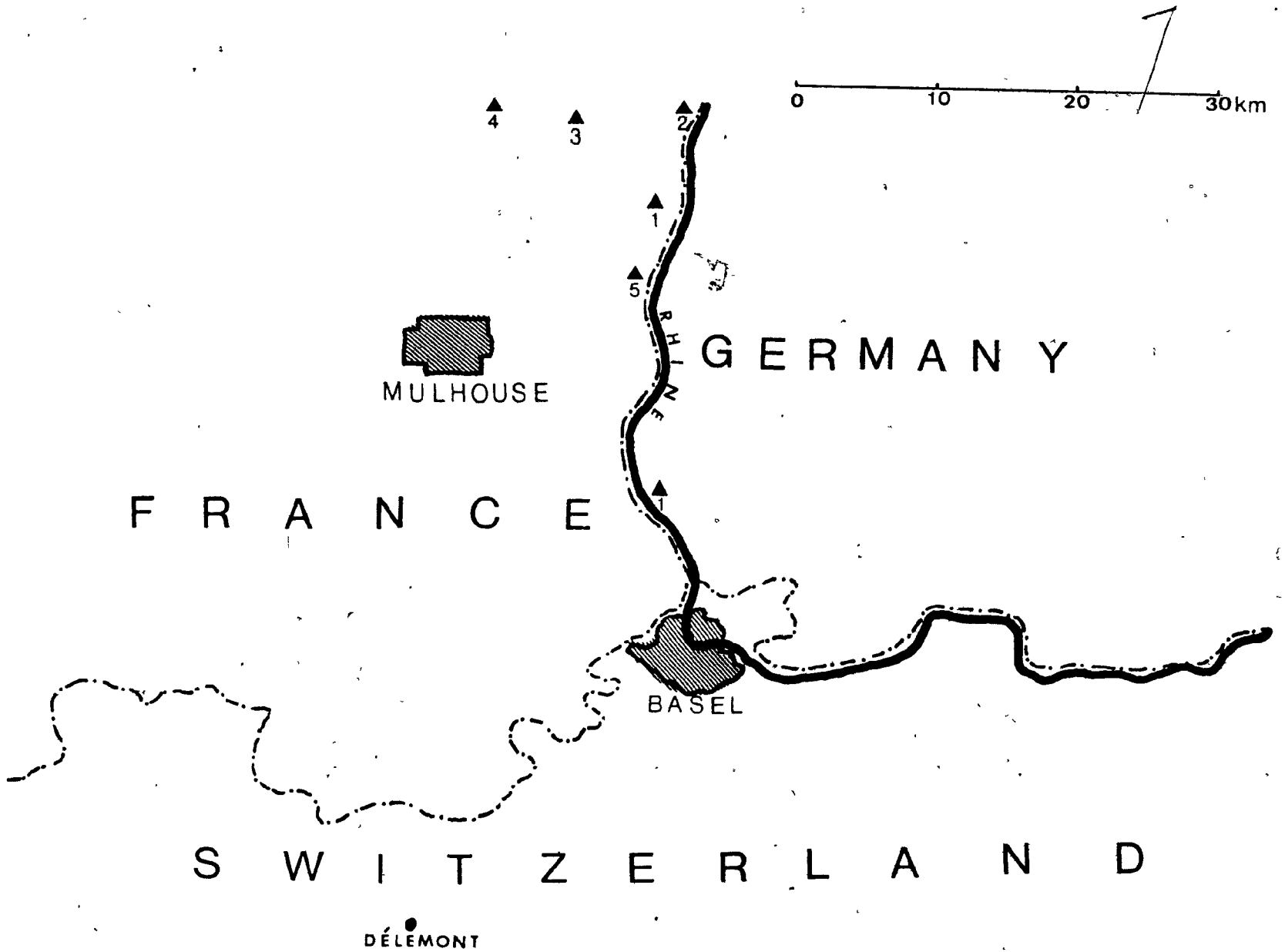

U S S R
HUNGARY
Oradea
Crisul Ic
5
4
Cluj
T R A N S Y L V A N I A
Hanu
Conachi
R O M A N I A
Letea
Tulcea
Babadag
YUGOSLAVIA
W A L A C H I A
BUCURESTI
BULGARIA
0
100
200 km

Figure 6. Collection sites (▲) of rust fungi on Centaurea maculosa Lam. in France and Germany.


0
10
20
30km
4
3
2
1
5
RHINE
GERMANY
MULHOUSE
FRANCE
BASEL
SWITZERLAND
DÉLEMONT

Researchers from the C.I.B.C. had previously identified the populations of spotted knapweed at different sites in Austria, Hungary, and France as C. maculosa ssp. rhenana. C. maculosa ssp. micranthos was also present in one population in Hungary. These plants were all found to be diploid (2n=18) (D. Schroeder, pers. comm.). However, tetraploid plants (2n=36) have recently been identified from five collection sites in Hungary and Romania, including one Romanian site (RM-04) in which a rust collection was made during this survey (Watson, pers. comm.). This confirms that different forms of spotted knapweed overlap geographically in their distribution in Europe and that rust collections may have been made on these different forms of the weed during this study.

All collection sites were found in open, disturbed habitats such as roadsides, waste places, and quarries (Figure 7a,b), with the exception of one site in Hungary which was a natural forest steppe habitat (Figure 7c). As previously reported, it appears that spotted knapweed is exclusively a ruderal species in Europe and is not found in cultivated fields (Hegi 1912). Spotted knapweed infestations were small (rarely exceeding 2 hectares) and they generally corresponded to the magnitude of soil disturbance in the site. Spotted knapweed was commonly found growing solitary, but occasionally grew in small groups of four or five plants. The degree of coverage was estimated to be less than 5% of the total area at all sites except for two sites in Austria (OS-14

Figure 7. Typical European habitats of Centaurea maculosa Lam. :

a) Spotted knapweed plants (arrow) with a scattered distribution in a recently abandoned quarry (collection site OS-07).

b) Spotted knapweed plants (arrow) on a slope of a vineyard (collection site OS-03).

c) Spotted knapweed plants (not seen in the picture) with a scattered distribution in a natural forest-steppe habitat (collection site HG-04).

7a

7b

7c

Figure 7d. Typical European habitat of C. maculosa Lam.: Dense infestations of spotted knapweed plants in a recently disturbed field (collection site OS-14).

Figure 8. External morphological structure of a spotted knapweed plant along a roadside (collection site OS-07).

Figure 9. Rust pustules (arrow) on basal leaves of a spotted knapweed plant (collection site OS-07).


7d


8


9

and OS-15) where dense stands of spotted knapweed seedlings were observed, suggesting recent soil disturbance at these sites (Figure 7d). Spotted knapweed was commonly associated with plant communities composed of other pioneer species such as Plantago lanceolata L., Carduus nutans L., Melilotus officinalis (L.)Desr., Anthemis cotula L., Echium sp., and Achillea sp..

The low density of spotted knapweed observed in most sites surveyed in Europe is an indication that biotic factors are playing a major role in regulating the population density of this weed . Spotted knapweed harbors a complex of natural enemies in its native range which contributes to the maintenance of low plant density (Schroeder 1977).

4.3.2 Rust Collection

A total of 106 rust collections were made on spotted knapweed (Table 4) with collections being made at each site surveyed except for one site in Hungary (HG-04) where no infected plants were found. These results indicate that spotted knapweed is probably attacked by rust fungi throughout its native range.

The prevalence of the rust disease was observed to be low with fewer than 10% of the plants being infected at each site. The scattered distribution and scarcity of spotted knapweed may have influenced the incidence and spread of the

disease in a location. The "underpopulation" of specialized pathogens caused by low host frequency may partly explain why serious epidemics in natural mixed vegetation are rare (Zadoks 1972). The severity of the disease was also observed to be low on most plants sampled with usually only the basal leaves of the bolted plants covered with uredinia (Figure 9). Occasionally, seedlings were found to be infected by rust. Reactions of spotted knapweed plants in the field to the rust usually varied from 1 to 4 according to Stakman's scale. Differences in host reactions were often observed within a population of spotted knapweed plants at one location. Rust collections which appeared most virulent on spotted knapweed (reaction types 3 and 4) were obtained from the following sites: OS-07, OS-09, OS-14, OS-17, OS-18, RM-04, and RM-05. Rust collections from the Rhine Valley appeared to be less virulent than collections made in eastern Europe.

The incidence of rust disease on spotted knapweed populations in Europe has also been observed to be low during previous surveys (D. Schroeder, pers. comm.). Although actual data on disease development in natural ecosystems are scarce, it is generally agreed that in the center of diversification where pathogens and host plants have commonly co-evolved over an extended period of time, disease, while being ever present, rarely approaches epidemic levels (Burdon and Schattock 19 ; Harlan 1976; Knott 1972; Segal et al. 1980; Zadoks 1972). In these situations, both host and pathogen have reached a

dynamic equilibrium in which the disease does not threaten the host, and the host supports the pathogen indefinitely (Harlan 1976). This may explain the low incidence and severity of rust disease that is probably prevalent throughout the native range of spotted knapweed.

There is no general consensus amongst plant pathologists concerning the effect of diseases on the genetic composition of plant populations in natural communities. Van der Planck (1975) and Nelson (1979) have emphasized the significance of horizontal (or field) resistance in preventing destructive disease incidence in the epicenters of host-pathogen co-evolution. Burdon (1978) attributed the typically low levels of diseases in natural plant communities to their heterogenic composition. Others have postulated that race-specific, oligogenic mechanisms of protection are also present in indigenous ecosystems (Browning 1974; Browning et al. 1977). As for the nature of the pathogen, Knott (1972) and Van der Planck (1975) contended that disease in natural ecosystems favors the evolution of relatively low virulence in the pathogen. Others argued that virulent and aggressive forms do develop in indigenous ecosystems (Moseman 1971; Zhukovsky 1959).

More extensive field studies are needed to understand the dynamics of rust disease on spotted knapweed in its native range. Nevertheless, field data from the present survey suggest that the nature of virulence in the rust pathogen may be distinctly diversified.

CHAPTER V. SCREENING OF RUST COLLECTIONS

5.1 Introduction

The screening of rust collections made in Eastern and Central Europe was undertaken to determine the most virulent strain on North American spotted knapweed. The lack of adaptation on spotted knapweed of the six rust collections previously tested in 1981 may have resulted from genetic variations in spotted knapweed in Europe which precludes certain biotypes of the rust from utilizing forms differing from those on which it evolved. Hasan (1972, 1981) has reported that rust collections made on Chondrilla juncea L. in southern Europe differed greatly in virulence to the Australian forms of the weed and that specific strains had to be selected for certain forms of the weed. The extended survey and more intensive collecting on spotted knapweed in Europe should have increased the chance of finding more virulent strains of the rusts attacking this weed .

5.2 Material and Methods

After their arrival at the quarantine facility at

Macdonald College, collections were divided into two portions: one portion was used immediately for inoculation on spotted knapweed seedlings and the other portion was retained as a herbarium specimen for later taxonomic observations. Collections which had limited development of uredinia were kept as herbarium specimens only.

Seeds of spotted knapweed from different locations in western North America were planted in pots (10.0 cm diameter, 8.5 cm high) filled with Pro-Mix, approximately 3 to 5 weeks prior to inoculation. The seedlings were thinned to a maximum of four per pot. Plants were grown in a controlled environment cabinet with 20°C ± 2°C day and 15°C ± 2°C night temperature, daylength of 15 hours, and light intensity at plant level of 320 ± 10 $\mu E\ sec^{-1}\ m^{-2}$. Plants were fertilized once every two weeks with a complete commercial formulation of $20N:20P_2O_5:20K_2O$ at a rate of 2.5g of fertilizer per liter of water.

Inoculation procedure

a) Urediniospores were hydrated for three hours. Spores or small leaf pieces with uredinia were placed on veighing paper situated on a moistened filter paper inside a petri dish sealed with parafilm.

b) The plants were moistened with an atomizer spray bottle containing sterile distilled water.

c) Hydrated urediniospores were transferred by finger or with the aid of a spatula to the leaf surface.

d) Inoculated leaves were gently rubbed with the finger to spread the urediniospores.

e) Inoculated plants were then lightly sprayed with sterile distilled water and enclosed separately in a polyethylene bag and incubated in the dark for 24 hours at 23°C.

f) Bags were removed and inoculated plants transferred to a controlled environmental cabinet with 20°C ± 2°C day and 15°C ± 2°C night temperature, daylength of 15 hours, and light intensity at plant level of 320 ± 10 $\mu E\ sec^{-1}\ m^{-2}$ from cool white fluorescent tubes supplemented with incandescent lamps. Relative humidity in the growth cabinet ranged from 60% to 80%.

Disease assessment

The reaction type of each plant was assessed 21 days after inoculation using the rating system of 0 to 4 developed for other rusts by Stakman *et al.* (1962) as follows:

Rust Rating System

Infection Type	Host Reactions
0	Immune: No uredinia nor other symptoms
0;	Nearly immune: No uredinia, but hypersensitive spots present.
1	Very resistant: Uredinia minute, surrounded by distinct necrotic areas.
2	Moderately resistant: Uredinia small to medium, usually in green islands surrounded by a chlorotic or necrotic border.
3	Moderately susceptible: Uredinia medium in size, no necrosis but chlorotic areas may be present. Coalescence of uredinia is infrequent.
4	Very susceptible: Uredinia large, and often coalescing. No necrosis, but chlorosis may be present.

Urediniospores were collected by tapping infected leaves over a petri dish and by cutting off leaves infected with uredinia. This material was stored at 4°C for a maximum of one month or until subsequent inoculation. The inoculum of the rust collections was increased on spotted knapweed using the same inoculation procedure described above.

5.3 Results and Discussion

All rust collections were received within 18 days of the date of collection at the plant quarantine facility. Sixty-three collections had sufficient uredinia development for inoculation on seedlings of spotted knapweed. Results of pathogenecity tests of these rust collections are summarized in Appendix 1. All main geographic regions surveyed , the Rhine Valley, southeastern Austria, western Hungary, and western Romania gave rust collections virulent to North American spotted knapweed. In total, 48 rust collections representing 21 European sites were pathogenic on at least one population of spotted knapweed.

Variations in host response were observed between and within populations of spotted knapweed inoculated with different rust collections. However, it is difficult to determine if these variations in reaction type are solely the consequences of genetic factors of resistance in the host population. Many inoculations resulted in an immune host response (reaction type 0), and could be attributed to the loss of inoculum viability from the time the collections were made in Europe to when they reached the plant quarantine facility by postal shipment (approximately 15 days). Prasad (1947) reported that urediniospores of Puccinia carthami Cda, a closely related rust , had lost their viability

within three weeks at room temperature (25-35°C). It was later observed using light microscopy, that teliospores were present in pustules of more than 20 of the rust collections made on spotted knapweed in Europe. The presence of teliospores in early summer on leaves of spotted knapweed in Europe suggests that they represent the oversummer stage of the rust cycle where dry conditions, as in the Steinfeld area, prevail throughout the summer. These spores will become dormant for a certain period of time and will require repeated periods of wet-dry cycles before they germinate (Petersen 1974). These two factors, the presence of teliospores and the decrease in inoculum viability, combined with a relatively low initial inoculum may have decreased the probability of obtaining infection on seedlings of spotted knapweed. Also, because of the very low initial inoculum, germination tests were not performed on these collections.

The inoculation procedure, incubation period and controlled environmental conditions used in these experiments were selected according to the optimum conditions reported for Puccinia carthami and many other rusts (Coulson 1967; Hasan 1972; Prasad 1947; Stakman et al. 1962).

When infection occurred on inoculated seedlings of spotted knapweed, uredinia developed 11 to 13 days after inoculation. Infection types 2 or 3 were often recorded on seedlings of the different spotted knapweed populations.

Occasionally, infection types 1 or 4 were observed on few individuals. These variable results were rather consistent for the different rust collections coming from different sites and also between collections coming from the same site. No hypersensitive response (reaction type 0;) was observed on any of the inoculated leaves. The range of host response in the North American populations of spotted knapweed appears to be similar to that which was observed in the field survey.

The most virulent rust collections are listed in Table 5. These 14 rust collections produced infection type 3 or 4 on spotted knapweed seedlings. The ratio of susceptible (reaction types 3 and 4) and resistant (infection types 1 and 2) plants to each collection is tabulated. Both resistant and susceptible plants were found for 10 of these virulent rust collections. One collection from Romania, RM-05-b, was highly virulent on spotted knapweed as indicated by a susceptible host response in all 14 infected seedlings from three populations. The uredinia produced by this collection were very large and developed rapidly on the surface of the leaf. This rust collection was chosen to be used for all subsequent testing . The other collections were kept viable by inoculating them every month on seedlings of spotted knapweed. The rust collection RM-05-b was later identified as Puccinia centaureae DC . Taxonomic studies on this rust collection are reported in Chapter VIII, page 117.

Table 5. List of the most virulent rust collections on populations of spotted knapweed.

Rust Collection	Host Population	No. of susceptible plants (infection type[a] 3 or 4) / No. of resistant plants (infection type 1 or 2)
[illegible]-31-c	SK-15,-17	11/7
OS-05-a	SK-11,-17	10/4
OS-05-f	SK-11,-17	2/5
OS-07-g	SK- 1,-17	11/2
OS-09-c	SK-11,-16,-17	5/1
OS-11-d	SK-11,-14,-17	10/6
OS-12-d	SK-17	12/5
OS-14-a	SK-17	19/9
OS-14-c	SK-15,-16,-17	10/1
OS-15-a	SK-11,-15,-17	12/15
RM-05-b	SK- 4,-8,-17	14/0

[a] Rating system:

0 Immune: No uredinia nor other symptoms.

0; Nearly immune: No uredinia, but hypersensitive spots present.

1 Very resistant: Uredinia minute, surrounded by distinct necrotic areas.

2 Moderately resistant: Uredinia small to medium, usually in green islands surrounded by a chlorotic or necrotic border.

3 Moderately susceptible: Uredinia medium in size, no necrosis but chlorotic areas may be present. Coalescence of uredinia is infrequent.

4 Very susceptible: Uredinia large, and often coalescing. No necrosis but chlorosis may be present.

CHAPTER VI. HOST SPECIFICITY OF PUCCINIA CENTAUREAE

6.1 Introduction

Prior to the release of an exotic weed control organism in North America, it is necessary to demonstrate its host specificity. Therefore, once the pathogenicity of the rust collections to North American populations of spotted knapweed had been determined, the collection which appeared the most virulent, RM-05-b, was selected for subsequent host specificity testing. This rust collection was later identified as Puccinia centaureae DC. A prime concern of biological control is that the biocontrol agent must not attack any cultivated or ecologically important plants in the region in which it is to be released.

Different methods have been described; those which have been perfected for testing the safety of exotic organisms are mainly for insects (Dunn 1978; Goeden 1977; Zwolfer and Harris 1971). Wapshere (1974b) has developed a method, the centrifugal phylogenetic system, that is applicable to all organisms, including rust pathogens. This approach is based on the assumption that related plants are morphologically and biochemically more similar than unrelated plants. The

procedure is to test the agent on a sequence of plants from those most closely related to the weed species, progressing to successively more and more distantly related plants until the host range has been adequately circumscribed. As a further assurance of safety, Wapshere suggested that the crop plants whose mycological record is poorly known and the crop plants that, for climatic or ecological reasons have, not been exposed to the biological agent, should also be tested at the same time as crop plants related to the weed and crop plants attacked by related organisms. Harris and Zwölfer (1968) suggested that plants possessing similar secondary chemicals should also be tested. Wapshere (1974b) has discussed the biological principles supporting his testing method. He also recognized that certain limitations exist if only plant biochemistry is used for establishing the list of test plants (Wapshere 1983). He explained that in the case of insects attacking two related weeds, Echium species and Heliotropium species, there was a relationship between plant chemicals and host selection which could have confirmed the host specificity demonstrated by testing. However, in the case of the host specificity of Chondrilla juncea arthropods, only one species had a host selection that seemed to be related to known phytochemistry. There are yet no clear indications on the phytochemistry of the genera of the sub-tribe Centaurineae that would help in establishing a list of potential hosts for organisms attacking Centaurea sp. (Wagner 1977).

The centrifugal phylogenetic system as described originally by Wapshere remains the safest method for the determination of host specificity of biocontrol organisms. This method has been used effectively to demonstrate the safety of many biological control organisms and also the specificity of Puccinia chondrillina on skeletonweed (Hasan 1972; Wapshere 1975). This method was used in this study to determine the host range of an isolate of P. centaureae collected on spotted knapweed in Romania.

Biological specialization, as an adaptation of obligate parasites to live on definite host plants only, is explicitly manifested in rust fungi (Leppik, 1965). Rust fungi have evolved in interdependence with their hosts in the center of origin and genetic diversification of the latter. This concept, first expresssed by Dietel in 1904, has gained general acceptance (Flor 1955; Gaumann 1952; Leppik 1970; Savile 1971; Zhukovsky 1959). The range of host specificity of rust fungi enables them to attack different host species but at the same time to have highly specific interactions within their main host species (Leppik 1965).

Host specificity tests have been found effective for selecting safe biocontrol agents since host transference has not occurred among organisms used for biological control of weeds in North America (Huffaker 1973).

6.2 Materials and Methods

The host specificity of P. centaureae was examined using the centrifugal phylogenetic system. The sequence of test plants was as follows:

Testing sequence	Plants to be tested
1	Populations of Centaurea maculosa
2	Other Centaurea sp.
3	Other members of the sub-tribe Centaureinae Dumort.
4	Other members of tribe Cynareae
5	Representatives of other tribes of Asteraceae Family.
6	Selected species of major economic importance from other Families.

The test plants are listed under the names used in Flora Europaea (Tutin et al. 1976). The tribes of the Asteraceae family were classified according to Cronquist (1955, 1977). Classification of the genera within the Cynarae was taken from Dittrich (1977) but the sub-tribes of Cynareae were not elevated to tribal rank as proposed by Dittrich. Cronquist (1977) did not agree with elevating these related sub-tribes

to tribal rank. Sub-genera and sections of the sub-tribe Centaureinae have been treated according to Tutin et al. (1976).

The plants were grown in pots 10 cm diameter and 14.4 cm high filled with Pro-Mix. They were inoculated at the juvenile stage (4 to 6 weeks old) by following the inoculation procedure described in section 5.2. Fresh inoculum of P. centaureae was previously harvested from spotted knapweed plants. The number of individuals of each plant species tested varied depending on availability of seed collections and germination rate. A maximum of four plants were inoculated in each pot. Each inoculation of tests plants was accompanied by 3 to 4 inoculated spotted knapweed plants. All inoculated plants were incubated and transferred to a controlled-environment cabinet as described in section 5.2. Disease assessment was performed 21 days after inoculation.

6.3 Results

Host specificity tests were first carried out by inoculating wild and cultivated Centaurea sp. closely related to spotted knapweed, followed by species of other genera of the four sub-tribes of Cynareae, namely; Centaureinae , Carduinae, Carlininae and Echinopsidinae. Afterwards, members of other tribes of Asteraceae were tested followed by representatives of other plant families which are economically

important in North America. Results of host specificity are summarized in table 6.

None of the plant species outside the Cynareae tribe, inoculated with the isolate of P. centaureae, became infected. These non-host species include 12 major economic crop plants and 13 species representing 9 tribes of Asteraceae. Two tribes, the Mutiseae and Vernoneae, were not represented in the testing because of unavailability of seeds. Members of these two tribes are distributed in the southern Hemisphere, with the exception of some Vernonia sp. which are found in North America. These two tribes have no species of economic importance (Cabrera, 1977; Jones 1977).

The experimentally determined host range of P. centaureae was confined to four genera of the sub-tribe Centaurinae, namely; Amberboa, Carthamus, Centaurea and Cnicus. All inoculations on spotted knapweed plants that accompanied each test resulted in infection and subsequent development of many uredinia on the leaf surface 11 days after inoculation indicating that all tests were performed under optimum conditions for rust infection.

P. centaureae was found to be pathogenic on 25 of the 52 species or subspecies of Centaurea tested. Susceptible Centaurea species showing reaction types 3 and 4 were found in the sub-genera Acrolophus, in which Centaurea maculosa belongs, and also in sub-genus Jaceae, Phalolepis, and Cyanus. The

Table 6. Results of host specificity tests of *Puccinia centaureae* DC.

	Number of plants inoculated	Infection type [a]
Family Asteraceae		
Tribe Cynareae		
Sub-tribe Centaureinae		
Sub-genus Acrolophus		
Section Maculosae		
Centaurea maculosa Lam.		
North American populations		
SK-1 (Québec, Canada)	6	3
SK-2 (Spokane, Wash., USA)	6	2
SK-5 (Québec, Canada)	4	2,3
SK-6 (Pullman, Wash., USA)	9	3,4
SK-8 (Montana, USA)	13	2,3
SK-10 (Oregon, USA)	3	1,2
SK-11 (Québec, Canada)	19	2,3
SK-14 (Québec, Canada)	17	2
SK-15 (Montana, USA)	10	3,4
SK-16 (Montana, USA)	5	3
SK-17 (Montana, USA)	155	2,3,4
SK-32 (California, USA)	5	3,4
European populations		
Austria - 1	5	2,3,4
Austria - 2	5	3,4
Austria - 3	5	3
Austria - 4	5	3
Czechoslovakia - 1	5	3
Czechoslovakia - 2	5	2,3
Czechoslovakia - 3	5	3
Czechoslovakia - 4	5	2,3
Czechoslovakia - 5	5	1,2,3
Czechoslovakia - 6	5	2

Table 6. (Continued)

	Number of plants inoculated	Infection type[a]
Sub-genus Acrolophus		
Section Maculosae		
Centaurea maculosa Lam.		
European populations (cont'd)		
West Germany - 1	5	2
West Germany - 2	5	3,4
Hungary - 1	5	2,3
Hungary - 2	5	3
Hungary - 3	5	3
Hungary - 4	5	3
Hungary - 5	5	3
Hungary - 6	2	2
Romania - 4	5	3
Centaurea vallesiaca (DC.) Jordan	3	0,3
Section Arenaria		
Centaurea arenaria Bieb. ex Willd.	2	0
Section Cylindracea		
Centaurea diffusa Lam.		
DK-1 (Wash., USA)	3	0,0;,1
DK-5 (Oregon, USA)	3	0;,2
DK-6 (Idaho, USA)	5	0,2
DK-9 (Wash., USA)	1	2
DK-10 (Cal., USA)	4	0;,1,2
DK-21 (Babadag, Romania)	7	0,0;,2,3
DK-22 (Cal., USA)	16	2,3,4

Table 6. (Continued)

	Number of plants inoculated	Infection type[a]
Sub-genus Acrolophus (cont'd) Section Paniculata		
C. paniculata L.	11	0
Section Calcitrapa		
C. calcitrapa L.	10	0,0;
Sub-genus Seridia		
C. aspera L.	9	0
C. napifolia L.	7	0
C. sonchifolia L.	2	0
Sub-genus Solstitiaria		
C. solstitialis L.		
Sol-1 (Danemark, Europe)	4	0
Sol-2 (Trinity Co., California,USA)	8	0,0;,1
Sol-3 (Loomis,Co.,California, USA)	7	0,1
C. melitensis L.	7	0;,1
C. sulphurea Willd.	2	0
C. eriophora L.	3	0
C. diluta Aiton	2	0
Sub-genus Phalolepis Section Phalolepis		
C. alba L.	8	0,2,3
C. alba ssp. deusta Ten.	6	0,2,3

Table 6. (Continued)

	Number of plants inoculated	Infection type [a]
Sub-tribe Centaureinae (cont'd)		
Sub-genus Jacea		
Section Jacea		
C. jacea L.	6	0
Jac-1 (Rhine Valley, R.F.A.)	2	0
Jac-2 (California, USA)	7	0,0;,1
Jac-3 (Quebec, Canada)	10	0,2
C. jacea L. ssp. amara	1	1
Section Fimbriatae		
C. decipiens Thuill. ssp. decipiens	7	0
C. macroptilon Borbas	10	0,1
C. microptilon Gren. et Gondron	12	0,0;,1
Section Nigrescentes		
C. nigrescens Willd.	4	0
Section Lepteranthus		
C. debeauxii Gren. et Gondron ssp. nemoralis (Jordan) Dostàl	8	0,0;,1
ssp. thuillieri Dostàl	3	1
C. nigra L.	1	0
Nig-1 (Denmark)	5	0,0;
Nig-2 (Cap Breton, Canada)	4	0
Nig-3 (Nova Scotia, Canada)	8	0
C. nigra L. ssp. rivularis	2	0

Table 6. (Continued)

	Number of plants inoculated	Infection type[a]
Sub-genus Jacea		
Section Lepteranthus (cont'd)		
C. phrygia L.	8	1,2
C. phrygia L. ssp. carpatica (Pore) Dostál	4	0,1
C. uniflora Turra		
ssp. uniflora	3	3
ssp. nervosa (Willd.) Bonnier & Layens	6	0;,1,2
C. pectinata L.	5	0;,2
Sub-genus Psephellus		
C. dealbata Willd.	5	0
Sub-genus Cyanus		
C. montana L.	4	0
C. depressa Bieb.	1	0
C. cyanus L.		
Cyn-1 (Belgium)	1	2
Cyn-2 (Finland)	1	3
Cyn-3 (California, USA)	3	3
Cyn-4 (Besançon, France)	3	0;,3
Sub-genus Lopholoma		
Section Aegialophila		
C. aegialophila Wagenitz	8	0
Section Lopholoma		
C. alpestris Hegetschw.	4	0

Table 6. (Continued)

	Number of plants inoculated	Infection type[a]
Sub-genus Lopholoma		
Section Lopholoma (cont'd)		
C. scabiosa L.	10	0,0;
ssp. uniflora	5	0
ssp. alba	4	0
Section Orientales		
C. atropurpurea Waldst. & K.T.	4	0
Section Acrocentron		
C. ornata Willd.	6	0
C. grbavacensis (Rohlena) Stoj. & Acht.	4	0;,0
Sub-genus Centaurea		
C. ruthenica Lam.	1	0
C. africana Lam.	3	0
Species not classified in Flora Europeae		
C. americana Nutt.	6	0
C. ferox Desf.	1	0
C. involucrata Desf.	6	2,3
C. macrocephala Puschk. ex. Willd.	7	0
C. muricata L.	1	0

Table 6. (Continued)

	Number of plants inoculated	Infection type[a]
Centaurea species not classified in Flora Europeae (Cont'd)		
C. simplicaulis Boiss. & Huet	4	0
C. virgata Lam.	4	0
Tribe Cynareae		
Sub-tribe Centaureinae		
Acroptilon repens (L.) DC.	13	0
Amberboa moschata (L.) DC.	3	1,2,3
ssp. *suaveliens*	5	0,2
Carthamus tinctorius L.		
Cultivar "DART"	2	0;
"GILA"	2	2
"VC-41"	2	2
"VFR"	2	1
Carthamus lanatus L.	8	0
Cheirolophus sempervirens (L.) Pomel	8	0
Cnicus benedictus L.	7	1
Cnicus gnaphaloides (Ajr.) Bertd.	8	0
Crupina crupinastrum (Moris) Vis.	4	0
Cyanopsis muricata (L.) Dostàl	19	0
Leuzea centauroides (L.) J. Holub	8	0
Mantisalca salmantica (L.) Briq. & Cavillier	10	0

Table 6. (Continued)

	Number of plants inoculated	Infection type[a]
Tribe Cynareae		
Sub-tribe Centaureinae (cont'd)		
Serrulata tinctoria L.	5	0
Volutaria lippii (L.) Maire	7	0
Sub-tribe Carduinae		
Arctium minus Bernh.	2	0
Arctium lappa L.	4	0
Carduus nutans L.	10	0
Cirsium arvense (L.) Scop.	8	0
Cirsium vulgare (Savi.) Ten.	16	0
Cousinia hystrix L.	4	0
Cynara scolymus L. (c.v. Green Globe)	18	0
Cynara cardunculus L.	12	0
Galactites tomentosa Moench	16	0
Jurinea alata	9	0
Notobasis syriaca (L.) Cass.	10	0
Onopordum arabicum auct., non L.	6	0
Picnomon acarna (L.) Cass.	11	0
Ptilostemon casabonae (L.) Greuter	2	0
Saussurea albescens Hook & Thoms	13	0
Silybum marianum (L.) Gaertner	10	0

Table 6. (Continued)

	Number of plants inoculated	Infection type[a]
Tribe Cynareae (cont'd) Sub-tribe Carlininae		
Carlina vulgaris L.	10	0
ssp. *stenophylla*	10	0
Xeranthemum annuum L.	14	0
Xeranthemum superbissimum	5	0
Sub-tribe Echinopsidinea		
Echinops banaticus Rothel ex. Sharader	4	0
Echinops ritro L.	11	0
Tribe Eupatorieae		
Ageratum haustonianum Miller	2	0
Tribe Inuleae		
Filago vulgaris Lam.	8	0
Inula helenium L.	11	0
Tribe Heliantheae		
Helianthus annuus L.	11	0
Tagetes erecta L.	6	0
Tagetes patula L.	4	0

Table 6. (Continued)

	Number of plants inoculated	Infection type[a]
Family Asteraceae (cont'd)		
Tribe Astereae		
Aster chinensis L.	3	0
Tribe Anthemideae		
Chrysanthemum maximum Ramond	11	0
Tribe Senecioneae		
Arnica montana L.	1	0
Senecio bicolor (Willd.) Tod. ssp. *cineraria* (DC.) Chater	12	0
Tribe Calenduleae		
Calendula officinalis L.	12	0
Tribe Arctotideae		
Gazania rigens (L.) Gaertner	9	0
Tribe Cichorieae		
Lactuca sativa L.	12	0
Crop plants from other families		
Family Solanaceae		
Solanum tuberosum L.	16	0

Table 6. (Continued)

	Number of plants inoculated	Infection type[a]
Crop plants from other families (cont'd)		
Family Apiaceae		
Daucus carota L. var. *sativa* DC. (c.v. Tournon)	20	0
Family Rosaceae		
Fragaria ananassa Duch.	11	0
Family Fabaceae		
Glycine max Merr.	12	0
Medicago sativa L.	20	0
Family Linaceae		
Linum usitatissimum L.	16	0
Family Brassicaceae		
Brassica napus L.	20	0
Brassica campestris L.	8	0
Family Chenopodiaceae		
Beta vulgaris L. var. *conditiva*	20	0

Table 6. (Continued)

	Number of plants inoculated	Infection type[a]
Crops plants from other families (cont'd)		
Family Poaceae[b]		
Avena sativa L.	12	0
Hordeum vulgare L.	12	0
Triticum aestivium L.	4	0

[a] Rating System:

0 Immune: No uredinia nor other symptoms.

0; Nearly immune: No uredinia, but hypersensitive spots present.

1 Very resistant: Uredinia minute, surrounded by distinct necrotic area.

2 Moderately resistant: Uredinia small to medium, usually in green islands surrounded by a chlorotic or necrotic border.

3 Moderately susceptible: Uredinia medium in size, no necrosis but chlorotic areas may be present. Coalescence of uredinia is infrequent.

4 Very susceptible: Uredinia large, and often coalescing. No necrosis, but chlorosis may be present.

species tested in sub-genus Solstitiaria and Lopholoma had resistant or immune responses. Only immune species were found in species of sub-genus Seridia and Psephellus. Centaurea species not classified in Flora Europeae and on which P. centaureae was pathogenic include C. involucrata and C. virgata. The only species native to North America, C. americana, was immune to the rust.

The species of Centaurea tested have been classified according to their response to rust infection (Table 7). Seven species, including C. maculosa, were fully susceptible . Few resistant plants were found in these species. A resistant host response (reaction type 1 and 2) was recorded on 10 additional species of Centaurea. This means that P. centaureae was able to infect and produce a second generation of urediniospores on juvenile plants of 17 Centaurea species. All other species were nearly to completely immune to rust infection and no uredinia were produced on these plants.

P. centaureae was inoculated on spotted knapweed plants from 12 localities of eastern and western parts of North America. The majority of plants from these populations were susceptible with only few moderately resistant individuals. Similar responses were observed in inoculated spotted knapweed plants from 19 European sites. There were no appreciable differences in host responses among all these populations of spotted knapweed. The lack of hypersensitive response on all inoculated leaves of spotted knapweed is

Table 7. Response of Centaurea species to Puccinia centaureae DC.

Immune Response		Nearly Immune
Infection Type[a] 0		Infection Type 0;
C. aegialophila Wagn.	C. nigra L.	C. calcitrapa L.
C. africana Lam.	C. nigra L. ssp. rivularis	C. cyanus L.
C. alba L.	C. nigrescens Willd.	C. debauxii
C. alpestris Hegetschw.	C. ornata Willd.	ssp. nemoralis
C. americana Nutt.	C. paniculata L.	C. diffusa
C. arenaria Bieb. ex Willd.	C. phrygia L.	C. grbavaçensis (Rohlena)Stoj.
C. aspera L.	ssp. carpatica(Pore)Dostàl	& Acht.
C. atropurpurea Waldst.& K.T.	C. ruthenica Lam.	C. microptilon
C. dealbata Willd.	C. scabiosa L.	C. nigra
C. calcitrapa L.	ssp. scabiosa	C. pectinata L.
C. debauxii Gren.& Gondron	ssp. uniflora	C. scabiosa
ssp. nemoralis (Jordan)Dostàl	C. simplicaulis Boiss.& Huet	C. solstitialis
C. decipiens Thuill. ssp. decipiens	C. solstitialis L.	C. uniflora Turra
C. depressa Bieb.	C. sonchifolia L.	ssp. nervosa (Willd.)Bonnier
C. diffusa Lam.	C. sulphurea Willd.	& Leyens
C. diluta Aiton	C. vallesiaca (DC.)Jordan	
C. eriophora L.		
C. ferox Desf.		
C. jacea L.		
C. macrocephala Pusck.ex Willd.		
C. macroptilon Borbas		
C. microptilon Gren.& Gondron		
C. montana L.		
C. muricata L.		
C. napifolia L.		

Table 7. (Continued)

Resistant response Infection type 1 and 2	Susceptible response Infection type 3 and 4
C. alba	C. alba
C. alba ssp. deusta Ten.	C. alba ssp. deusta
C. cyanus	C. cyanus
C. debauxii ssp. nemoralis ssp. thuillieri Dostàl	C. diffusa
C. diffusa	C. involucrata
C. involucrata Desf.	C. maculosa
C. jacea	C. uniflora ssp. uniflora
C. jacea ssp. amara	C. vallesiaca
C. macroptilon	
C. maculosa Lam.	
C. melitensis L.	
C. microptilon	
C. phrygia	
C. phrygia ssp. carpatica	
C. pectinata	
C. solstitialis	
C. uniflora ssp. nervosa	
C. virgata Lam.	

[a] Rating system as described on page 84.

noteworthy.

P. centaureae was able to infect under controlled environment conditions, three species outside the genus Centaurea. The response to rust infection by Amberboa moschata (L.)DC. was from very resistant to moderately susceptible (reaction types 1 to 3). This species has also been previously named Centaurea moschata L. . Carthamus tinctorius L. showed a range of resistant response, from nearly immune to moderately resistant (reaction types 0; to 2). Cnicus benedictus L. was very resistant to the rust (reaction type 1). Carthamus lanatus L. and Cnicus gnaphaloides (Cyr.)Bertol. were immune to rust infection (reaction type 0).

6.4 Discussion

The host specificity study reveals that the isolate of P. centaureae, collected on spotted knapweed in Romania, is pathogenic on other Centaurea species and that its host range extends to three other genera of the sub-tribe Centaurinae that have never been reported to harbor this rust species. However, the rust isolate was found to be of very low virulence on many of these species as indicated by a nearly immune and highly resistant host response. This was particularly true for Centaurea sp. belonging to sub-genus Jaceae and Solstitiaria, and also for Carthamus tinctorius and

Cnicus benedictus. It could be possible that this extended host range represents a controlled-environment phenomenon and may not represents the true field host range. According to Yarwood (1959), there are many cases of successful artificial inoculation of plants with pathogens which have not been found associated with these plants in nature. Although such a phenomenon could also be explained by the fact that pathogen and hosts did not come in contact in nature or that field observations have not been sufficiently intensive, it is suspected that predisposition, under controlled environmental conditions, is playing a major role in extending the host range of P. centaureae. The principal predisposing features of controlled-environment experiments may not be known precisely. However, the fact that plants in a growth cabinet are usually more liberally watered and fertilized , and the conditions are optimum for pathogenesis during the experiment, as compared to field environment which varies constantly, may predispose the plants to disease.

Van der Planck (1975) and Nelson (1979) have indicated that in ecosystems, horizontal (or field) resistance in nature is of major importance and that hypersensitivity is a rare event (Nelson 1979). Nelson cited the example of Solanum species and the blight fungus which co-evolved in Mexico and where no tuber-bearing Solanum species were immune or hypersensitive when exposed to the pathogen under natural field conditions in that country. Others have

however presented different opinions and recognized that hypersensitivity and vertical resistance do have a role to play in natural ecosystems but only if they are "backstopped" by field resistance (Browning 1974, 1981; Browning et al. 1977; Segal et al. 1980). The consistency of immune, hypersensitive and highly resistant responses, under controlled environmental conditions, in many wild Centaurea species does not reflect the genetic diversity, with respect to host response, that might be expected from natural host species. Moreover, in the host specificity test as well as the screening of rust collections, spotted knapweed populations showed a range of host responses but no hypersensitivity. It is then questioned if these resistant species would actually harbor this rust isolate in Europe. On the other hand, since the rust was able to infect and produce secondary inoculum on some of these resistant species, it is suspected that the rust could possibly transfer and adapt itself to these potential hosts in absence of its natural host in the plant community. The plasticity and broad adaptability of this rust, as recognized in this study, would explain the number of different variants of P. centaureae reported in Europe (Gaumann 1959; Guyot 1967; Savile 1970a).

The geographical distribution of Centaurea in Europe seems to give an indication of the potential host range of the rust isolate. Taxonomic relationships between these species are of little value since both host and non-host species were

found in the same sub-genus such as Acrolophus. By examining the distribution of Centaurea species as reported in Flora Europaea (Tutin et al. 1976), it was found that all the Centaurea species tested which are distributed outside the native range of spotted knapweed or found in alpine habitats were immune to the rust isolate. The species on which the rust isolate was pathogenic are in part or totally distributed inside the native range of the target weed. Because of geographic isolation, Centaurea species which are distributed outside the native range of spotted knapweed or found at high altitudes may have never been exposed to this variant which has become specialized on its main host species. However, these species have been reported to harbor other variants of P. centaureae (Guyot 1967) and these variants have probably become adapted to their hosts in the same way as the variant on spotted knapweed.

It is interesting to note that some species such as Centaurea jacea and C. scabiosa which are probably growing in the same habitat as spotted knapweed in Europe (Hegi 1912) were very resistant to the rust isolate. This may indicate further specialization of the spotted knapweed rust since these species have also been reported to harbor other variants of P. centaureae in Europe (Guyot 1967). This would mean that only six species of Centaurea, listed as susceptible in Table 8, may represent natural hosts of the P. centaureae isolate.

Species from three related genera of Cynareae were found to be potential hosts of P. centaureae, namely Amberboa moschata, Carthamus tinctorius and Cnicus benedictus. A similar host range sequence has also been observed in insects feeding pattern which reflects the botanical relationship of these genera (Zwölfer 1970). Dittrich (1977) has grouped together these four genera on the basis of palynological, morphological, and anatomical fruit characters. It is suggested that plant biochemistry in these genera is an important factor in host recognition, especially for specialized parasites. Rust fungi react to a complex of chemical substances and have a very intimate biochemical relationship with their hosts (Heath 1982). Some investigators believe that host ranges may point to the close evolutionary relationships between the hosts (Leppik 1967;Savile 1979; Watson 1972). However, since Amberboa, Carthamus and Cnicus have never been reported to host P. centaureae in Europe and were very resistant to the P. centaureae isolate, evolutionary relationships among the different rusts found on these species and P. centaureae could be suggested. Savile (1970a) has classified under the same lineage, the Puccinia centaureae - P. laschii Lineage, a complex of brachycyclic rusts on Cynarae, including species on Centaurea, Cnicus and Carthamus. Amberboa is closely related to Centaurea, with A. moschata often named Centaurea moschata . This species was found to be susceptible to P. centaureae in this study, and these results may be of taxonomic value for a proper classification of this

species.

The specificity study revealed that the P. centaureae isolate is highly virulent on spotted knapweed and thus is of great interest for the biological control of this weed. However, the fact that this rust can also attack three economically important species is of major concern. Bachelor button (Centaureae cyanus) and sweet sultan (Amberboa moschata) were fully susceptible to P. centaureae. These two species are ornamentals, but are rarely grown in large quantities. Four cultivars of cultivated safflower (Carthamus tintorius) were infected but were considered as resistant. Safflower is a minor oil crop grown in southern U.S.A.. Because this crop represents a potential host for P. centaureae, further studies were conducted to determine the levels of resistance in different cultivars and to estimate the impact of the spotted knapweed rust on this crop .

CHAPTER VII. SAFFLOWER EXPERIMENTS

7.1 Introduction

The host specificity study revealed that P. centaureae can infect cultivated safflower, Carthamus tinctorius, under controlled environmental conditions but only resistant host response was observed in the four safflower cultivars tested. Further investigations were undertaken to determine the degree of resistance amongst several safflower cultivars. Safflower cultivars were also used in order to determine the degree of virulence of three rust collections.

Because of the economic importance of safflower and some morphological similarities between the safflower rust, Puccinia carthami , and P. centaureae , the rust disease of safflower has been briefly reviewed in this section. The taxonomy of these rusts will be discussed in section 8.4.

Safflower has been cultivated since ancient times in many countries of the world, but it is considered today as a minor oilseed crop in terms of total production and world trade (Weiss 1971). Its production in North America has declined sharply since 1960 because of lower demand for safflower oil (Weiss 1971). In U.S.A., it is mainly grown in

California with about 80,000 acres (A. Weisker, pers. commun.). Safflower is no longer grown on a commercial basis in Canada (Weiss 1971).

More than 12 diseases have been recorded on safflower with the most economically important one being a rust disease caused by P. carthami, which had been estimated to cause an average annual loss of 5 percent between 1950 and 1960 in U.S.A. (Weiss 1971). As a result of the macrocyclic-autoecious nature of P. carthami, safflower rust has two distinct pathological phases; a seedling phase resulting from invasion of young seedlings by basidiospores produced by soil-borne or seed-borne teliospores and a foliage phase resulting from invasion by pycniospores and urediniospores (Sackston 1953; Schuster 1956; Schuster and Christiansen 1952). The seedling phase can cause a serious reduction in stands while the foliage phase is believed to cause little, if any, loss of yield (Zimmer and Jensen 1970; Zimmer and Urie 1968a).

Resistant cultivars have been developed and proven successful as a means of controlling the disease for both phases of the rust cycle (McCain 1963; Zimmer and Urie 1968b; Zimmer et al. 1968). Resistance in safflower to the seedling phase is closely allied to foliage phase resistance and probably physiologically and genetically related (Zimmer 1962; Zimmer and Urie 1967; Zimmer and Urie 1969). A seedling rust test has been an efficient means of screening for foliage rust

resistance since only one cultivar, Nebraska 115, has been found to be resistant to the seedling phase but susceptible to the foliage phase (Zimmer et al. 1968). Resistance is conditioned by dominant or partly dominant genes and has been found in safflower introductions from foreign countries (Ashri 1971; McCain 1963; Zimmer and Urie 1968b, 1969). Several races of the rust fungus have been identified in the U.S.A. through host differential tests (Thomas 1955, 1958; Zimmer 1963).

P. carthami is found wherever safflower is grown and has been recorded on five wild species of Carthamus; C. glaucus Bieb., C. lanatus L. C. oxyacantha Bieb., C. palaestinus Eig., and C. arborescens L. (Conners 1943; McCain 1963). Savile (1944) and McCain (1963) reported that P. carthami is capable of infecting bachelor button, Centaurea cyanus L., in the greenhouse but only small to medium-sized pustules, surrounded by chlorotic areas, are formed.

7.2 Material and Methods

7.2.1 Response of safflower cultivars to P. centaureae

7.2.1.1 Experiment 1

Seventeen cultivars of safflower, one collection of spotted knapweed (Sk-17) and one collection of wild safflower

(Carthamus lanatus) were tested for their reaction to P. centaureae. Six plants of each test species and cultivar were inoculated at the four leaf stage with hydrated urediniospores that were collected from spotted knapweed one month before inoculation and stored at 4° C. The inoculation procedure described in section 5.2 was followed. Each 8.5 cm x 10 cm pot contained three plants and all plant parts were inoculated. Three control plants of each test species and cultivars were sprayed with sterile water only and incubated along with the inoculated plants in plastic bags for 24 hrs in the dark at 23° C. All plants were transferred to a growth cabinet with 20° ±2°C day and 15° ± 2°C night temperature, daylength of 15 hrs, and light intensity at plant level of 320 ± 10 $\mu E\ sec^{-1}\ m^{-2}$. Relative humidity in the cabinet ranged from 60% to 80%. Pots were randomly distributed on four carriers that were rotated every two days in the growth cabinet. Plants were watered as needed and fertilized as described in section 5.2. Disease rating was assessed 21 days after inoculation using the scale of 0 to 4 as described in section 5.2.

7.2.1.2 Experiment 2

Following the screening of safflower cultivars, an experiment was established to determine the influence of growth stage of susceptible and resistant safflower cultivars, and spotted knapweed on the pathogenicity of P. centaureae.

Based on disease reactions obtained in experiment 1, two susceptible cultivars (14-5 and Oleic Leed) and three resistant cultivars (S-208, S-541 and N-10) were chosen and inoculated with urediniospores of RM-05-b collection.

Twelve plants of each cultivars were first inoculated at the four leaf stage. Each 14.5 x 15 cm pot contained four plants and all plant parts were inoculated following the same procedure described in section 5.2. Three control plants of each cultivar were maintained. Twelve spotted knapweed plants inoculated at the four-leaf stage accompanied the safflower cultivars. All plants were incubated in the same way and in identical controlled-environment conditions as described for experiment 1. Pots were randomly distributed on four carriers that were rotated every two days in the growth cabinet. Plants were watered as needed and fertilized as described in section 5.2. Disease rating was assessed 21 days following inoculation.

The plants were allowed to grow until the heading stage (60 days old). Three out of four plants in each pot were re-inoculated with fresh inoculum of urediniospores of P. centaureae harvested from spotted knapweed plants. All leaves of these plants were inoculated following the procedure described in section 5.2. The other plant in each pot served as a check. The plants were incubated and transferred to the same growth cabinet conditions as described above. Disease reaction was assessed 21 days after re-inoculation.

7.2.2 Pathogenicity of three European rust collections on five safflower cultivars

Two rust isolates (OS-07-g and OS-11-d) collected on spotted knapweed in southern Austria and RM-05-b isolate from western Romania were tested for their virulence on five safflower cultivars (14-5, Oleic Leed, S-208, S-541, and N-10). Inoculum of each rust isolate was increased on spotted knapweed in separate growth chambers. The urediniopores were collected two weeks before inoculation and stored in petri dishes at 4°C.

For each rust collection, nine plants of each safflower cultivar were inoculated at the six-leaf stage. Each 8.5 cm x 10 cm pot contained three plants and only the first pair of true leaves were inoculated following the same procedure described in section 5.2. Three control plants sprayed only with sterile water were maintained for each cultivar. Nine spotted knapweed plants were also inoculated at the six-leaf stage.

All plants were incubated and placed in a growth cabinet as described in all previous experiments. Pots were randomly distributed on four carriers that were rotated every two days in the growth cabinet. Plants were watered as needed and fertilized as described in previous experiments. Disease rating was assessed 21 days after inoculation.

7.3 Results

7.3.1 Response of safflower cultivars to P. centaureae

7.3.1.1 Experiment 1

Responses of safflower cultivars and spotted knapweed plants to P. centaureae are listed in Table 8. Pustules developed on all plants of spotted knapweed 11 days after inoculation. All inoculated leaves of spotted knapweed showed a susceptible reaction to the rust isolate (Figure 18). The latent period on safflower was delayed and varied from 13 days in the most susceptible cultivar (14-5) to 15 days in the resistant cultivar (RH-3). This period was taken as the first day after inoculation on which any lesions produced secondary inoculum. Other workers have also characterized latent period in the same way (Shaner and Powelson 1971; Zadoks 1961). With the exception of Oleic Leed cultivar, there were no differences in infection type between the six inoculated plants of the same cultivar. A range of host response was recorded among cultivars of safflower and spotted knapweed as illustrated in Figures 10 to 17. Cotyledons of safflower cultivars were usually more susceptible than the first pair of true leaves (Figure 11). A hypersensitive reaction, as indicated by necrotic flecks and absence of uredinia, was observed on the first pair of true leaves of six cultivars; S-

Table 8. Response of safflower cultivars to P. centaureae DC.

	Latent Period (days)	Infection type[a]		
		Cotyledons	True leaves	
			1st-2nd	3rd-4th
Safflower cultivar				
S-541		—	0;	0;
S-208		—	0;	0;
RH-3	15	—	0;	1
VFR-1	14	1	0;	1
DART	15	1	0;	1
S-400	14	1-2	1	1
S-291	13	2	1	1
US-10	14	—	1	1
VC-41	14	—	0;	2
GILA	14	—	1	2
FRIO	14	2	1	2
N-10	13	3-4	1	2
PH	14	3	1	2
P1	14	3	2	2
PCOy	12	—	2	1
OLEIC LEED	13	—	1-3	2
14-5	13	3	3	3
Spotted knapweed				
SK-17 (Montana, USA)	11	—	3-4	3-4
Carthamus lanatus L.		0	0	0

— Cotyledons dead before showing symptoms (same for control plants).

[a] Described on page 109.

FIGURE 10. Different infection types (Stakman scale) on the first true leaf of safflower cultivars and spotted knapweed, 21 days after inoculation with P. centaureae.

a: Control leaf of safflower cultivar N-10.

b: Infection type 0; on safflower cultivar S-208. Only few necrotic flecks developed (arrow). Nearly immune response.

c: Infection type 1 on safflower cultivar N-10. Uredinia minute, surrounded by necrotic areas. Very resistant response.

d: Infection type 2 on safflower cultivar PCO_y. Uredinia small to medium in size with chlorotic border. Moderately resistant response.

e: Infection type 3 on spotted knapweed (SK-17). Uredinia medium in size. Moderately susceptible response.

f: Infection type 4 on spotted knapweed (SK-17). Uredinia large and coalescent. Very susceptible response.

FIGURE 11. Reaction of safflower cultivar N-10 to P. centaureae. Infection type 3 and 1 on cotyledon and first leaf respectively, 21 days after inoculation.

FIGURE 12. Reaction of safflower cultivar VC-41 to P. centaureae. Infection type 2 on 4th leaf, 21 days after inoculation. Moderately resistant response.

FIGURE 13. Reaction of safflower cultivar S-541 to P. centaureae. Necrotic flecks (arrow) on first leaf, 21 days after inoculation. Hypersensitive response.


10


11


12

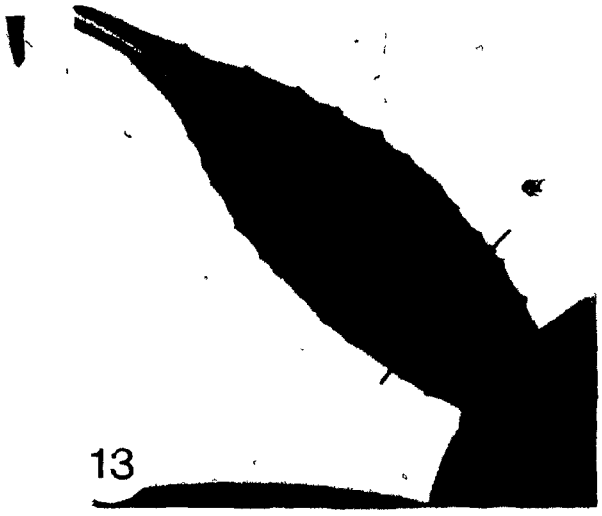

13

FIGURE 14. Reaction of safflower cultivar GILA to P. centaureae. Infection type 1 on 2nd leaf, 21 days after inoculation. Very resistant response.

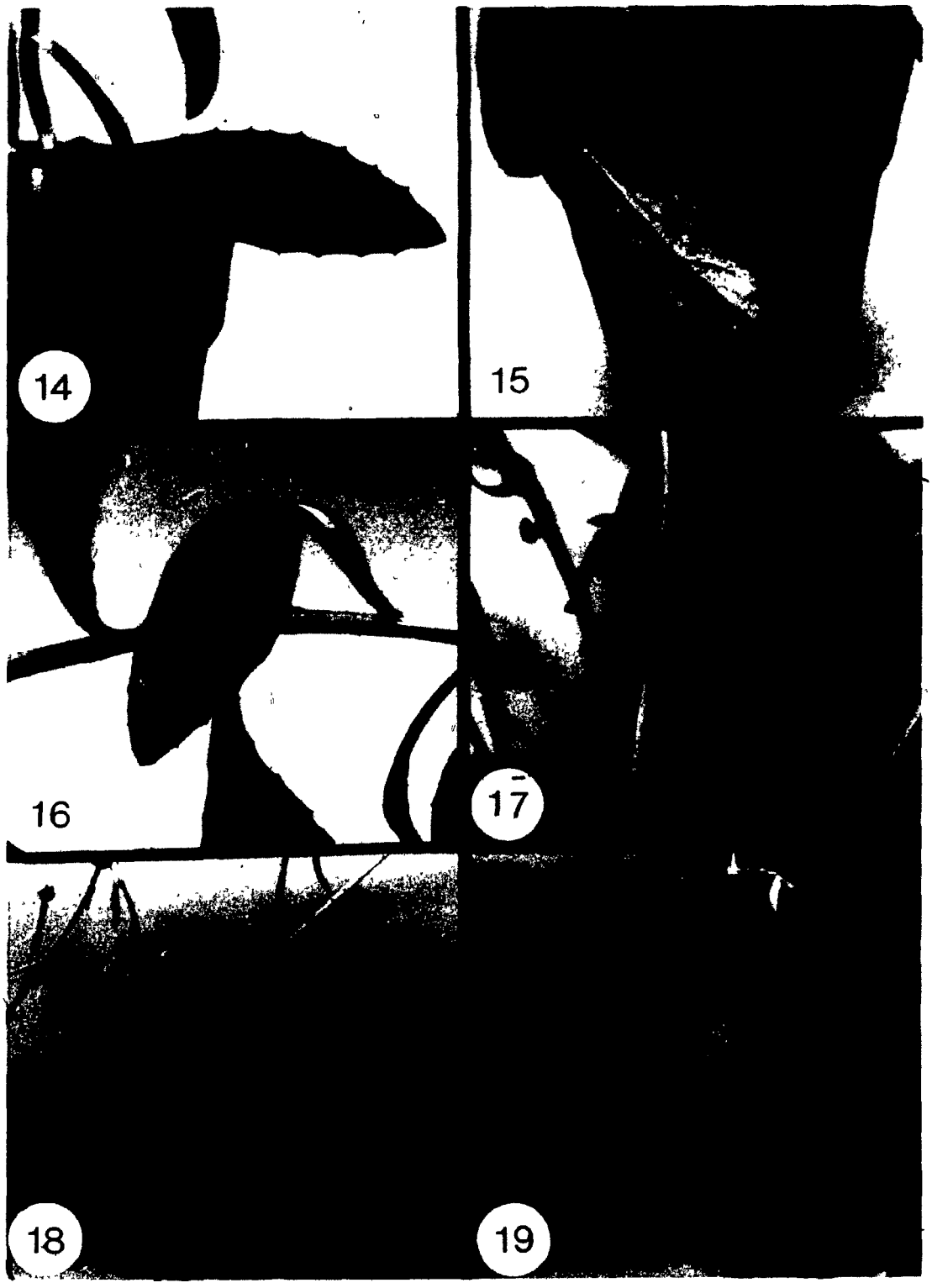
FIGURE 15. Reaction of safflower cultivar OLEIC LEED to P. centaureae. Infection type 1 on 1st leaf, 21 days after inoculation. Very resistant response.

FIGURE 16. Reaction of safflower cultivar PH to P. centaureae. Infection type 2 on 4th leaf, 21 days after inoculation. Moderately resistant response.

FIGURE 17. Reaction of spotted knapweed (SK-17) to P. centaureae. Infection type 4 on 4th leaf, 21 days after inoculation. Very susceptible response.

FIGURE 18. Reaction of spotted knapweed (SK-17) to P. centaureae after re-inoculation at heading stage. Large uredinia developed on stems and leaflets (arrow), 21 days after re-inoculation.

FIGURE 19. Reaction of safflower cultivar N-10 to P. centaureae after re-inoculation at heading stage. Small-sized uredinia developed on lower leaf of the plant. Very resistant response.


14
15
16
17
18
19

541, S-208, RH-3, VFR-1, Dart and VC-41 (Figure 13). Few small-sized uredinia developed on the 3rd and 4th leaf of these cultivars, except for cultivars S-541 and S-208 which had a nearly immune reaction on all leaves.

Thirteen cultivars had a resistant response (infection type 1 or 2) on their first two pairs of true leaves inoculated with the rust isolate. However, cotyledons of three of these cultivars N-10, PH and P1 were moderatly susceptible. Cultivar 14-5 was moderately susceptible but fewer uredinia were produced on the leaves compared to spotted knapweed plants and their development was delayed by approximately two days. Infection types from 1 to 3 were recorded on different plants of Oleic Leed. Wild safflower (Carthamus lanatus) which was included in the screening, was immune to rust infection. No symptoms developed on control plants of safflower and spotted knapweed.

7.3.1.2 Experiment 2

Seedlings of the five safflower cultivars inoculated with P. centaureae gave the same response as in experiment 1 (Table 9). Cultivar 14-5 was moderately susceptible and Oleic Leed still showed a range of infection type from 1 to 3. Cultivar N-10 was very resistant and cultivars S-208 and S-541 were nearly immune to rust infection. All inoculated

Table 9. Influence of growth stage on pathogenicity of P. centaureae on safflower cultivars and spotted knapweed.

	Four-leaf stage		Heading stage		Flowering
	Latent Period (days)	Infection type[a]	Latent Period (days)	Infection type[a]	Disease severity
Safflower cultivar					
S-208		0;		0	No symptoms
S-541		0;		0	No symptoms
N-10	14	1	16	2-3	Only bottom leaves infected
14-5	14	3	16	1-2	Only bottom leaves infected.
OLEIC LEED	13	1-3	16	1-3	Bottom half of the plant infected.
Spotted knapweed					
SK-17 (Montana,USA)	11	4	12	4	All plant parts infected including stems, leaflets and pedicels.

[a] Described on page 109.

seedlings of spotted knapweed were fully susceptible. No symptoms developed on the control plants.

Safflower plants of cultivars S-208 and S-541 that were originally inoculated at the 4-leaf stage and subsequently re-inoculated at heading stage did not develop any pustules nor necrotic flecks on their leaves. Re-inoculated plants of cultivars N-10 and 14-5 showed pustules but only the lower leaves became infected (Figure 18). Pustules on lower leaves of mature plants of cultivar N-10 were usualy larger in size than those recorded on seedlings. The uredinia on 14-5 were however smaller than those observed at seedling stage. Oleic Leed plants had few uredinia on the lower half of the plants and their size did not vary from those recorded on seedlings. All plants of spotted knapweed were very susceptible at the heading stage with many large pustules developing on all inoculated parts including leaflets, stems and flower pedicels (Figure 19).

7.3.2 Pathogenicity of three European rust collections on five safflower cultivars

Responses of five safflower cultivars to rust collection OS-07-g and OS-11-d did not differ from those recorded for rust collection RM-05-b (Table 10). Infection types assessed on these five cultivars inoculated with rust

Table 10. Reaction of the first pair of true leaves of five safflower cultivars and spotted knapweed to three rust collections.

	Infection type of rust collection		
	OS-07-g	OS-11-d	RM-05-b
Safflower cultivar			
14-5	3	3	3
OLEIC LEED	1 - 3	1 - 3	1 - 3
S-208	0;	0;	0;
S-541	0;	0;	0;
N-10	1	1	1
Spotted knapweed			
SK-17 (Montana, USA)	3 - 4	3 - 4	3 - 4

[a] Ratingsystem:

0 Immune: No uredinia nor other symptoms.

0; Nearly immune: No uredinia, but hypersensitive spots present.

1 Very resistant: Uredinia minute, surrounded by distinct necrotic areas.

2 Moderately resistant: Uredinia small to medium, usually in green islands surrounded by a chlorotic or necrotic border.

3 Moderately susceptible: Uredinia medium in size, no necrosis but chlorotic areas may be present. Coalescence of uredinia is infrequent.

4 Very susceptible: Uredinia large, and often coalescing. No necrosis, but chlorosis may be present.

collection RM-05-b were identical to those recorded on the same cultivars in experiment 1 and 2. Cultivar 14-5 was moderately susceptible to all three collections. Cultivars S-208 and S-541 were nearly immune to all rust collections . The range of infection types from 1 to 3 on Oleic Leed was also observed for the two rust collections OS-07-g and OS-11-d. Infection type 1 was recorded on cultivar N-10 for all three rust collections. The rust collections were highly virulent on spotted knapweed seedlings as indicated by an infection type of 3 or 4.

7.4 Discussion

7.4.1 Response of safflower cultivars to P. centaureae

7.4.1.1 Experiment 1

The results indicate that different levels of resistance to the P. centaureae isolate exist among safflower cultivars. The different infection types, especially hypersensitivity, assessed in these cultivars suggests that vertical resistance may be involved. Vertical resistance is usually identified by a hypersensitive response of the host to the pathogen (Hooker 1967; Nelson 1979). Such reaction is a characteristic of a plant resistant to an infecting pathogen in an incompatible plant-pathogen relationship (Kiraly 1980).

It is characterized by the presence of necrotic flecks at the infection site. The early collapse and death of the host cells at the site prevents the further growth of the fungal hyphae (Robinson 1976). Such reaction has been recorded on six safflower cultivars tested in this experiment. Intermediate level of resistance in seedlings (reaction type 1 to 3) is also generally vertical or race-specific as demonstrated for certain rusts (Samborski and Dyck 1966, 1976). This resistance does not prevent colonization but reduces the rate of spread of the pathogen. Vertical resistance implies resistance to some pathogen isolates and not to others and is usually simply inherited (Van der Planck 1968). Resistance to all isolates of the pathogen is called horizontal and is often polygenically inherited. Many debates and discussions have emanated since Van der Planck first defined these two concepts (Ellinghoe 1981; Nelson 1978; Robinson 1976; Van der Planck 1982). It is not the author's intention to discuss in more detail the genetics of plant resistance.

It is interesting to note that similar levels of resistance have been observed in safflower cultivars inoculated with safflower rust, Puccinia carthami (Zimmer 1963). When tested against different races of P. carthami, infection types on seedlings inoculated with urediniospores varied from 0; to 4 among safflower cultivars. The sources of seedling rust resistance in safflower have also often been of a hypersensitive nature (Zimmer 1965; Zimmer et al. 1968).

Immune response of wild safflower (Carthamus lanatus) to races of P. carthami has been reported (McCain 1963; Zimmer et al. 1968). Resistance to safflower rust has been identified as vertical or race-specific and in many cases involves a single dominant gene pair (McCain 1963; Zimmer and Urie 1968b; Zimmer et al. 1968). The nature of safflower resistance to P. centaureae resembles in many respects the resistance to P. carthami. More studies are needed on the genetics of host resistance in safflower to both rusts. Since both rust species infect safflower, there is a possibility of crossing the two rusts and studying the inheritance of morphological characters as well as pathogenicity.

The P. centaureae isolate used in this study appears to be less virulent than P. carthami on the cultivars tested in this experiment. Nine cultivars tested are known to be susceptible to the foliage phase of P. carthami: US-10, P-1, Frio, N10, RH-3, Oleic Leed, and Gila (Registration of Safflower Germplasm, Crop Science). Of these cultivars, only Oleic Leed was susceptible to P. centaureae. The nature of resistance to P. carthami of cultivars 14-5 and PH was not found in the literature. Four cultivars S-208, S-400, S-296 and S-541, resistant to P. carthami and developed by SeedTec International Inc., at Woodland, California, were also resistant to P. centaureae. Cultivars Dart, PCO_y, and PCA were registered as resistant cultivars and were also found to be resistant to P. centaureae.

7.4.1.2 Experiment 2

Safflower plants of three cultivars inoculated at the 4-leaf stage with P. centaureae developed pustules on lower leaves but the infection did not appear to interfere with subsequent plant growth. Only minor chlorosis was observed on inoculated leaves of two other cultivars S-541 and S-208. Subsequent re-inoculation at heading stage suggests resistance within this crop to later infection. Symptoms developed only on basal leaves of the plants. None of the plants of cultivars S-208 and S-541 developed symptoms at this later stage. Spotted knapweed plants were all fully susceptible at both seedling and heading stages. This is of particular importance since ontogenic resistance will not be a rate reducing factor in the epidemiology of the rust. The infected leaves of spotted knapweed usually died one week before those on non-inoculated plants in the controlled environment conditions.

The latent period was longer in safflower than on spotted knapweed at the seedling stage and was lengthened for both safflower and spotted knapweed when re-inoculated at heading stage. The latent period was taken into account because it has been reported that resistance is manifested by a lenghtened latent period (Popular 1978; Van der Planck 1968).

Plants become generally more resistant to rust fungi as they get older (Hooker 1967). This adult-plant resistance was found to be of extreme practical importance in cereal crops (Allan et al. 1966; Dyck et al. 1966). Adult-plant resistance was observed to be present in safflower cultivars inoculated with P. centaureae in this study.

Zimmer and Urie (1968a) have reported that in order to cause significant reduction in yield of safflower, heavy infection of the foliage phase of P. carthami must occur before flowering and not be restricted to the lower leaves. These same authors have also indicated that in the regions where safflower is grown commercially in U.S.A., conditions conducive to a rapid build-up of the rust on the upper foliage do not normally occur and losses from foliage rust are minimized. Although free-moisture conditions may persist long enough in these regions to permit heavy rust infection on the lower leaves, it has been demonstrated that lower leaves of safflower can be removed or destroyed without significantly affecting yield components (Urie et al. 1968).

The threat P. centaureae poses to safflower is considered to be negligeable although further tests are needed to evaluate its impact under field conditions. It is important to know if this rust possesses a similar seedling phase as reported for P. carthami which can cause serious reduction in safflower stands. In this phase, the seed-borne teliospores germinate in the spring and produce basidiospores which in

turn germinate and invade young seedlings. P. centaureae has been observed to produce teliospores in early summer in Europe in order to withstand dry conditions but there is no indication as to the nature of its life cycle in winter conditions.

7.4.2 Pathogenicity of three European rust collections on five safflower cultivars

Differential host testing has been the only practical way to detect new races of P. carthami (Thomas 1955, 1958; Zimmer 1963). The procedure is to expose different isolates of the rust pathogen to several lines or cultivars of safflower. Differences in virulence between two isolates will be demonstrated if they produced different infection types on one or more of the safflower cultivars.

Three different rust collections from spotted knapweed at different locations in Eastern Europe did not differ in their virulence on five safflower cultivars. Each cultivar produced similar reactions to the three rust collections. The same levels of resistance to the P. centaureae RM-05-b isolate were found in the five safflower cultivars inoculated with the two other rust collections, OS-07-g and OS-11-d. However, the small number of safflower

cultivars included in the test reduced the chance of detecting any differences in virulence among the rust collections. The three rust collections were highly virulent on spotted knapweed and these three collections may represent in fact the same variant of P. centaureae.

In order to have a differential host response, the host and the pathogen must have co-evolved in an intimate relationship where for each gene for virulence that developed in the pathogen, a corresponding gene for resistance developed in the host. This gene-for-gene concept was first described by Flor (1955) in his work with flax rust. Safflower has never been reported as a natural host of P. centaureae. Although this rust was able to infect safflower under controlled environmental conditions, the rust does not appear to be well adapted to this species. This lack of adaptation would prevent any host-pathogen interactions that would have resulted in having specific races of the pathogen adapted to certain safflower cultivars as reported for safflower rust. It is however still unknown if different variants of P. centaureae would respond differently on safflower cultivars; a topic waiting for investigation.

CHAPTER VIII. TAXONOMIC STUDIES ON SPOTTED KNAPWEED RUST FUNGI

8.1 Introduction

The systematics of the Puccinia rusts associated with Centaurea and related genera has undergone considerable revision (Cummins 1978; Savile 1970a,b; Guyot 1967). Jacky (1899) first reported that Puccinia jaceae was a synonym of P. centaureae and that within P. centaureae there were two types, A and B, differentiated by the number and distribution of germpores of urediniospore. Since then, the classification of these two rusts has been treated differently by rust taxonomists. Gaumann (1959) grouped both P. jaceae and P. centaureae in the Puccinia hieracii lineage. Savile (1970a,b) classified these two species under different evolutionary lineages and gave varietal rank to material of both species on Centaurea species. Some taxonomists have adopted an extremely wide species concept for these rusts as exemplified by Cummins (1978) combining P. centaureae and P. carthami as one species, P. calcitrapa var. centaureae.

The use of spore morphology to delineate these rust species has been rendered difficult due to climatic adaptations and convergent evolution. These morphological variations have caused serious confusion in attempts to assign

rusts on Centaurea on the basis of teliospore characteristics (Savile 1970b). Urediniospores or urediniospores and teliospores together have been better sources of distinguishing morphological features that reflect evolutionary relationships (Savile 1970a, b). Urediniospores of P. jaceae are ellipsoid and flattened with two, rarely 3, superequatorial germpores and a more or less conspicuous circular area below each pore partly or wholly covered by fine echinulation which is characteristic of the P. dioicae - P. hieracii lineage (Savile 1970a). Urediniospores of P. centaureae and P. carthami are spherical and symmetrical with 3, rarely 2 or 4, equatorial germpores and are evenly echinulated except near the hilum where the spore wall is thickened, Based on these characteristics, both rust species have been grouped in the P. centaureae-P. laschii lineage (Savile 1970a).

Surface ornamentation, such as spines, warts and reticula, on the urediniospores and teliospores are sometimes of great taxonomic value and aid in the identification of rust fungi. Traquair and Kokko (1983) have observed differences in hilum (or spore attachment scar) surface of P. jaceae, P. centaureae and P. carthami. The hilum surface of P. jaceae was relatively smooth in contrast with the minutely and distinctly verrucose hilum of P. centaureae and P. carthami, respectively.

A study was undertaken to determine the taxonomic

position of rust fungi collected on spotted knapweed in Europe using light microscopy and electron microscopy on urediniospores and comparing the hilum surface of these rusts with P. carthami and one rust isolate collected from Centaurea jaceae.

8.2 Material and Methods

Light Microscopy

Light microscopy observations were made on 97 spotted knapweed rust collections from Europe. For each rust herbarium specimen, urediniospores were taken from two or three pustules and mounted in lactophenol. The slide was gently heated until the spores were turgid and thoroughly cleared. Observations were made with a Reicher Diavar microscope and measurements were made on 10 urediniospores. After a few random measurements, the entire slide was scanned to determine minimum and maximum spore sizes. The number and distribution of germpores were taken from 50 urediniospores chosen at random.

Scanning Electron Microscopy (SEM)

Observations were made on leaves of spotted knapweed

inoculated with rust collections RM-05-b, OS-07-g and OS-15-b. Leaves with uredinial pustules were cut 14 days after inoculation. Other rust specimens included in this study are: Puccinia carthami collected from safflower cultivar S-400 in a growth chamber at Macdonald College ; and one rust isolate collected on Centaurea jaceae at Ile Perrot, Quebec, Canada.

For each specimen, a leaf piece bearing uredinia was fixed following a modified procedure from Brown and Brotzman (1976). The samples were fixed in 5 ml of 2% OsO_4 (osmium) containing Kodak Photo-Flo 200 at 4°C for 24 hrs and then rinsed with three changes of distilled water over a 30 minute period. The samples were then treated with 5 ml of a saturated solution of thiocarbohydrazide for 1hr at room temperature. The samples were then rinsed with distilled water as described above and subsequently treated with 5 ml of OsO_4 for 1hr at room temperature. The samples were again rinsed with distilled water followed by a dehydration in an ethanol series (20, 40, 60, 80, 95, and 100% EtOH) at 20 minutes intervals. Finally, the samples were critical point dried and coated with gold before examination in a Cambridge Stereoscan 600 scanning electron microscope operating at 15 KeV.

8.3 Results

A comparison between spotted knapweed rust collections of mean lengths and widths of urediniospores

Table 11. Number, position and sizes of urediniospores of European rust collection on spotted knapweed.

Rust Collection	Number and position of urediniospores[a] observed with			Urediniospore measurements[b] (µm)		
	2 germpores	3 germpores	4 germpores	Width	$\bar{X}$	Length
FC - 01 - a	4 E[c]	36 E	10 E	(20.50-23.99)	22.22 X 24.23	(21.81-26.17)
FC - 01 - b	3 E	46 E	1 E	(20.71-26.17)	22.81 X 24.44	(21.81-26.17)
FC - 01 - c	2 E	48 E	0	(21.81-23.77)	22.74 X 23.99	(22.24-25.08)
FC - 02 - a	0	48 E	2 E	(23.77-27.26)	25.64 X 26.50	(24.86-28.13)
FC - 02 - b	0	50 E	0	(20.26-22.24)	21.26 X 22.77	(21.81-23.55)
FC - 03 - a	0	50 E	0	(21.15-25.74)	23.05 X 24.23	(21.81-25.30)
FC - 03 - b	0	49 E	1 E	(21.81-23.34)	22.16 X 22.90	(21.81-24.43)
FC - 03 - c	0	50 E	0	(19.19-21.81)	20.68 X 22.26	(21.16-23.77)
FC - 04 - a	0	49 E	1 E	(20.06-23.34)	21.88 X 23.40	(21.81-24.65)
FC - 04 - b	1 E	49 E	0	(20.94-22.46)	21.72 X 23.51	(21.81-24.86)
FC - 05 - a	0	50 E	0	(22.25-27.04)	24.32 X 24.88	(22.68-27.04)
GR - 01 - a	0	50 E	0	(20.06-23.34)	21.74 X 23.99	(22.90-24.65)
GR - 01 - b	0	50 E	0	(21.59-23.77)	22.40 X 23.55	(21.81-24.65)
GR - 01 - c	0	50 E	0	(21.37-23.12)	22.03 X 23.12	(21.81-24.86)
HG - 01 - b	1 E	49 E	0	(21.37-25.08)	23.05 X 25.45	(24.21-26.83)
HG - 01 - c	1 E	49 E	0	(21.81-23.99)	22.31 X 23.45	(21.81-26.17)
HG - 01 - e	0	50 E	0	(20.94-23.77)	22.70 X 24.25	(23.12-26.17)
HG - 02 - a	0	48 E	2 E	(23.12-24.65)	23.93 X 26.22	(25.08-27.92)
HG - 03 - a	2 E	48 E	0	(20.28-23.34)	21.50 X 24.01	(21.81-27.26)
OS - 01 - a	0	50 E	0	(20.07-23.55)	21.29 X 23.58	(21.37-24.86)
OS - 01 - b	0	50 E	0	(19.85-22.46)	21.13 X 22.81	(21.37-24.21)
OS - 01 - c	0	50 E	0	(20.72-22.25)	21.55 X 23.69	(21.81-24.86)
OS - 01 - d	0	50 E	0	(21.81-25.52)	23.55 X 25.13	(23.77-26.39)
OS - 01 - e	0	50 E	0	(19.63-23.77)	21.90 X 23.88	(21.81-25.52)
OS - 01 - f	0	50 E	0	(20.28-23.55)	21.70 X 23.45	(21.81-25.74)
OS - 01 - g	0	50 E	0	(18.97-21.81)	20.48 X 22.94	(20.94-24.43)
OS - 01 - h	0	50 E	0	(19.41-21.37)	20.50 X 23.51	(21.59-24.43)
OS - 01 - i	0	50 E	0	(19.41-22.46)	21.18 X 23.31	(21.59-24.65)
OS - 01 - j	0	50 E	0	(21.59-24.65)	23.31 X 20.34	(19.19-21.16)

Table 11. Number, position and sizes of urediniospores of European rust collection on spotted knapweed. (Continued)

Rust Collection	Number and position of urediniospores[a] observed with			Urediniospore measurements[b] (µm)		
	2 germpores	3 germpores	4 germpores	Width	$\bar{X}$	Length
OS - 02 - a	0	50 E	0	(19.63-23.99)	20.59 X 23.10	(21.81-26.17)
OS - 02 - b	0	50 E	0	(20.07-26.17)	21.07 X 23.21	(21.37-26.17)
OS - 02 - c	1 E	49 E	0	(21.37-24.43)	22.57 X 23.69	(21.81-26.17)
OS - 02 - d	1 E	49 E	0	(21.81-25.30)	23.60 X 25.06	(23.33-27.04)
OS - 02 - e	0	50 E	0	(18.54-22.90)	21.00 X 22.16	(20.94-23.55)
OS - 02 - f	1 E	49 E	0	(20.94-23.55)	22.16 X 22.12	(20.94-23.12)
OS - 02 - g	0	50 E	0	(19.63-23.77)	21.72 X 23.75	(21.81-25.30)
OS - 02 - h	0	50 E	0	(19.19-21.16)	20.24 X 22.51	(21.81-24.86)
OS - 03 - a	0	50 E	0	(20.50-22.90)	21.44 X 24.62	(22.25-28.13)
OS - 03 - b	0	50 E	0	(20.50-25.08)	23.05 X 26.02	(23.34-29.66)
OS - 03 - c	2 E	48 E	0	(25.52-29.44)	27.22 X 28.68	(27.04-30.53)
OS - 03 - d	1 E	49 E	0	(23.99-28.35)	26.04 X 27.20	(24.86-29.66)
OS - 03 - e	0	50 E	0	(21.37-23.55)	22.22 X 25.23	(24.21-27.48)
OS - 03 - f	0	50 E	0	(20.07-23.34)	21.88 X 25.39	(23.99-27.92)
OS - 04 - a	0	50 E	0	(20.72-25.08)	23.51 X 25.02	(22.68-27.26)
OS - 04 - b	0	50 E	0	(21.81-23.99)	22.62 X 25.32	(24.43-26.17)
OS - 04 - c	1 E	49 E	0	(21.81-23.34)	22.36 X 24.32	(21.81-26.39)
OS - 05 - a	0	50 E	0	(21.81-23.34)	22.14 X 24.50	(23.34-25.52)
OS - 05 - b	0	50 E	0	(22.25-28.57)	24.75 X 26.78	(24.43-28.57)
OS - 05 - c	0	49 E	1 E	(23.77-27.48)	25.28 X 27.20	(25.95-29.23)
OS - 05 - d	0	49 E	1 E	(21.81-25.95)	23.10 X 25.56	(22.90-27.48)
OS - 05 - e	0	50 E	0	(21.81-25.08)	23.38 X 25.43	(23.55-27.92)
OS - 05 - f	0	50 E	0	(22.90-23.77)	23.34 X 25.28	(24.21-26.83)
OS - 05 - g	0	50 E	0	(21.81-22.90)	23.07 X 24.18	(23.33-25.74)
OS - 05 - h	0	50 E	0	(22.24-23.99)	23.27 X 24.93	(22.90-26.39)
OS - 06 - a	0	50 E	0	(20.28-23.34)	21.61 X 24.14	(22.68-26.17)
OS - 06 - b	0	50 E	0	(21.81-24.43)	22.64 X 24.43	(23.99-25.52)
OS - 06 - c	0	50 E	0	(23.34-26.61)	24.54 X 25.47	(23.34-27.48)

Table 11 . Number, position and sizes of urediniospores of European rust collection on spotted knapweed. (Continued)

Rust Collection	Number and position of urediniospores[a] observed with			Urediniospore measurements[b] (µm)		
	2 germpores	3 germpores	4 germpores	Width	$\bar{X}$	Length
OS - 07 - a	0	50 E	0	(23.34-27.48)	25.47 X 26.85	(25.08-29.23)
OS - 07 - b	0	50 E	0	(22.03-26.83)	24.19 X 26.59	(25.30-27.92)
OS - 07 - c	0	50 E	0	(23.12-27.70)	24.73 X 26.48	(25.08-29.01)
OS - 07 - d	0	50 E	0	(21.81-24.65)	23.16 X 25.71	(24.43-28.13)
OS - 07 - e	0	50 E	0	(21.81-26.83)	24.58 X 26.39	(23.12-29.44)
OS - 07 - f	0	50 E	0	(22.03-26.17)	24.17 X 25.60	(23.99-26.83)
OS - 07 - g	0	50 E	0	(21.81-25.08)	23.62 X 25.08	(23.99-26.61)
OS - 08 - a	0	50 E	0	(19.63-23.34)	21.98 X 24.30	(22.68-25.08)
OS - 09 - a	0	50 E	0	(21.59-23.99)	22.87 X 24.80	(23.34-26.61)
OS - 09 - b	0	49 E	1 E	(21.81-24.86)	23.45 X 24.73	(23.34-26.60)
OS - 10 - a	2 E	46 E	2 E	(12.81-25.74)	23.49 X 25.50	(23.55-27.48)
OS - 10 - b	0	50 E	0	(23.34-25.95)	24.45 X 25.47	(24.21-26.39)
OS - 10 - c	0	50 E	0	(21.16-24.43)	23.18 X 24.97	(23.55-25.95)
OS - 10 - d	0	50 E	0	(23.34-27.48)	24.82 X 25.84	(24.65-27.48)
OS - 11 - a	0	50 E	0	(21.81-27.26)	24.12 X 25.95	(23.12-28.35)
OS - 11 - b	0	50 E	0	(18.54-21.81)	20.68 X 24.36	(23.34-26.61)
OS - 11 - c	0	50 E	0	(20.72-23.12)	22.07 X 23.69	(22.68-24.86)
OS - 12 - a	5 E	45 E	0	(21.81-24.43)	22.75 X 25.21	(21.77-26.17)
OS - 12 - b	3 E	47 E	0	(21.81-24.43)	22.64 X 25.17	(24.43-26.39)
OS - 12 - c	4 E	46 E	0	(22.68-24.43)	23.51 X 25.74	(24.21-28.57)
OS - 13 - a	0	50 E	0	(21.37-24.65)	22.99 X 24.86	(23.34-26.39)
OS - 14 - a	2 E	48 E	50 E	(21.81-23.99)	22.84 X 25.06	(23.99-26.39)
OS - 14 - b	0	50 E	0	(21.81-26.83)	23.62 X 25.08	(23.55-27.48)
OS - 14 - c	0	50 E	0	(21.81-25.30)	23.35 X 24.84	(22.68-26.17)
OS - 14 - d	0	50 E	0	(21.81-24.43)	23.55 X 25.40	(24.65-26.17)
OS - 14 - e	0	50 E	0	(22.03-25.08)	23.73 X 25.39	(23.12-26.61)
OS - 14 - f	0	50 E	0	(21.81-24.65)	23.10 X 24.80	(23.77-26.17)
OS - 15 - a	0	50 E	0	(22.46-26.39)	24.03 X 25.50	(24.43-26.83)
OS - 15 - b	50 SE[d]	0	0	(24.86-28.79)	26.35 X 27.02	(25.52-28.79)

Table 11. Number, position and sizes of urediniospores of European rust collection on spotted knapweed. (Continued)

Rust Collection	Number and position of urediniospores[a] observed with			Urediniospore measurements[b] (µm)		
	2 germpores	3 germpores	4 germpores	Width	$\bar{X}$	Length
OS - 15 - d	0	50 E	0	(21.81-26.61)	24.23 X 26.08	(24.21-27.48)
OS - 15 - e	0	50 E	0	(21.81-23.33)	22.38 X 24.25	(23.34-24.86)
OS - 16 - a	0	50 E	0	(21.81-24.43)	23.23 X 24.60	(23.77-25.08)
OS - 16 - b	0	50 E	0	(21.81-23.77)	22.86 X 25.28	(23.77-26.83)
OS - 16 - c	0	50 E	0	(22.03-24.86)	23.14 X 24.80	(23.77-25.74)
OS - 17 - a	0	50 E	0	(21.81-24.86)	23.58 X 24.73	(23.55-26.61)
OS - 17 - b	2 E	48 E	0	(21.37-23.77)	22.36 X 24.54	(23.34-26.39)
OS - 17 - c	2 E	48 E	0	(19.41-23.99)	21.57 X 25.19	(22.68-34.90)
OS - 18 - a	0	50 E	0	(21.81-22.90)	22.09 X 23.38	(21.81-23.99)
RM - 04 - a	0	50 E	0	(19.63-21.81)	20.76 X 21.98	(21.81-22.68)
RM - 05 - a	0	50 E	0	(20.07-23.34)	21.42 X 22.94	(20.07-24.68)
RM - 05 - b	0	50 E	0	(20.28-24.43)	21.88 X 23.69	(22.03-25.08)

[a] Total of 50 urediniospores observed per collection.

[b] Average of 10 spores with minimum and maximum values in parentheses.

[c] Equatorial position.

[d] Super-equatorial position.

revealed no consistent differences (Table 11). The dimensions of urediniospores in all collections were within the range of spore size reported in the literature for P. jaceae and P. centaureae (Table 2). With the exception of rust collection OS-15-b, urediniospores of all other collections were spherical and symmetrical in profile with 3, but also sometimes 2 or 4, germpores distributed equatorially (Figure 20). Urediniospores of rust collection OS-15-b, on the other hand, were shown to be broadly ellipsoidal with only two super-equatorial germpores (Figure 21). The urediniospores of this rust collection appeared to be slightly larger than the other collections as indicated by their minimum and maximum dimensions. The other rust collections which had few urediniospores with 2 germpores had them equatorially located and not super-equatorially as on urediniospores of rust collection OS-15-b.

Scanning electron microscopy has revealed differences in surface ornamentation among the four rust specimens studied. In these rusts, the spines were evenly distributed over the spore surface except for P. carthami which had a smooth area encircling the hilum at the base of the urediniospores. The surface of the hilum distinguished some of these rusts. The urediniospores of spotted knapweed rust collections, RM-05-b and OS-07-g, had a minutely verrucose hilum (Figure 22 and 23), in contrast with a distinctly verrucose hilum surface for P. carthami (Figure 24) and a

Figure 20. Phase-contrast light microscope photograph of a typical spherical urediniospore of rust isolate RM-05-b, showing the three equatorial germpores (g) and the hilum (h). X 500

Figure 21. Phase-contrast light microscope photograph of a typical ellipsoidal urediniospore of rust isolate OS-15-b, showing the two super-equatorial germpores (g) and the hilum (h). X 500

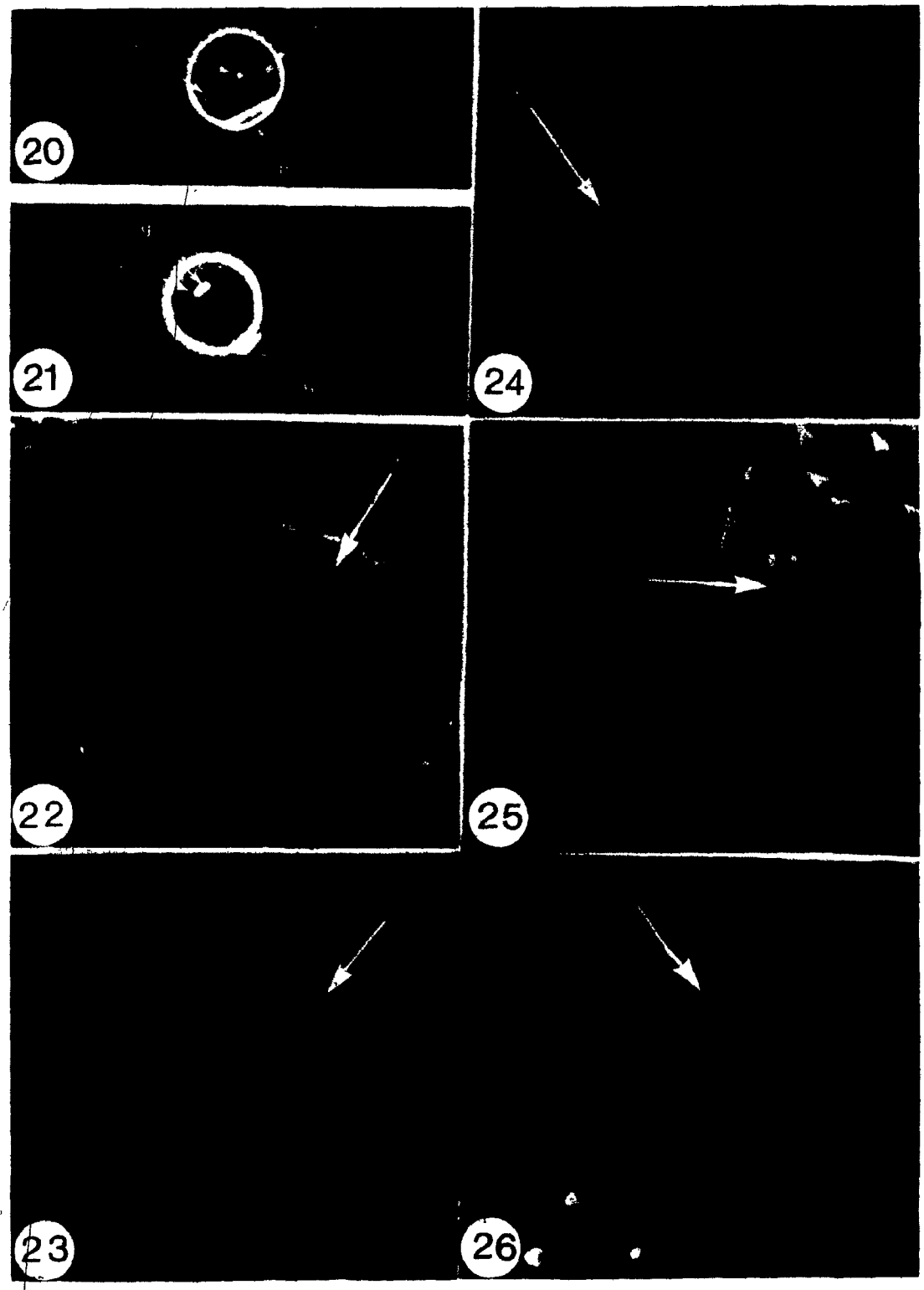
Figure 22. SEM photograph of a urediniospore of rust isolate RM-05-b on spotted knapweed, showing the minutely verrucose hilum (h). X 5000

Figure 23. SEM photograph of a urediniospore of rust isolate OS-07-g on spotted knapweed, showing the minutely verrucose hilum (h). X 5000

Figure 24. SEM photograph of a urediniospore of Puccinia carthami on safflower, showing the distinctly verrucose hilum (h). X 5000

Figure 25. SEM photograph of a urediniospore of rust isolate OS-15-b on spotted knapweed, showing the smooth hilum (h). X 5000

Figure 26. SEM photograph of a urediniospore of a rust isolate on Centaurea jacea, showing the smooth hilum (h). X 10,000


20
21
24
22
25
23
26

smooth hilum surface for the spotted knapweed rust OS-15-b (Figure 25) and the rust specimens collected on C. jacea (Figure 26). The urediniospores of this later rust isolate were previously observed with light microscope and these spores were found to be broadly ellipsoid in shape with only two super-equatorial germpores, which are similar to the urediniospores of spotted knapweed rust OS-15-b. Both of these isolates appear to be P. jaceae.

8.4. Discussion

This study has shown that the rust collections made on spotted knapweed in Europe have urediniospores typical of P. centaureae and are characterized by their spherical shape and the presence of three equatorial germpores. Only one collection, OS-15-b, had urediniospores typical of P. jaceae, which are ellipsoid in profile with two super-equatorial germpores. The few urediniospores from the other spotted knapweed rust collections with only two germpores had them equatorially located and not super-equatorial as on urediniospores of OS-15-b. Watson et al. (1981) reported that both urediniospores typical of P. centaureae and P. jaceae were frequently observed from pustules of a single leaf of diffuse knapweed (Centaurea diffusa). Although this has not been observed for spotted knapweed in this study, both rust species were found on different spotted knapweed plants originating from the same site (OS-15). This study showed that

spotted knapweed harbors both rust species in Europe, but P. centaureae is more frequent.

In addition to observations made with light microscopy, SEM study has also illustrated differences between the two rust species found on spotted knapweed. Rust collection OS-15-b had a typical smooth hilum compared with the minutely verrucose hilum of RM-05-b and OS-07-g. A similar rust to OS-15-b was observed from a leaf specimen of C. jacea and both are typical of P. jaceae. Safflower rust was readily differentiated from the other rusts by having urediniospores with a verrucose hilum. These findings agree with previous SEM studies made by Traquair and Kokko (1983). Whether these rusts should be recognized as distinct species is a matter for rust taxonomy authorities, but this study on urediniospores morphology provides further evidence that P. centaureae, P. jaceae and P. carthami represent distinct species as suggested by Savile (1970a, b).

Host specialization is traditionally an important adjunct to morphological descriptions to delineate rust species, but must be used with discretion. According to Savile (1970a), it is probable that a contributing factor to the confusion surrounding the systematics of this group of rust species has been a tacit assumption that a single host species, species group, or genus harbors only a single, genetically uniform parasite. Frequently, host plants such as members of the Cynareae accept more than one rust species and mixed infections are not uncommon (Savile 1970a, b). This

appears to be true for spotted knapweed and probably other Centaurea species. The host specificity test with one isolate typical of P. centaureae (RM-05-b) showed that this rust species is capable of infecting many Centaurea spp. and species from three other genera of Cynareae never reported before to harbor this rust. Host range studies under controlled environmental conditions are not always representative of true field host range. Nevertheless, the host range studies indicated a certain level of specificity considering that many species tested were highly resistant to the P. centaureae isolate, including species such as C. scabiosa, C. jacea, C. solstitialis, and C. nigra reported to harbor the rust in Europe (Guyot 1967). A strict comparison of host ranges between P. centaureae and P. jaceae would be difficult to make since these two rust species are highly variable, as indicated by the description of many variants, combined with the fact that many Centaurea spp. can harbor both rust species in Europe. It would be of particular interest to know if the variant of P. jaceae collected on C. jacea at Ile Perrot, in Québec and the variant of P. centaureae collected on C. nigra in Nova Scotia (Savile 1970b) are morphologically similar to the variants found in Europe and to compare their respective host ranges. These results could have a considerable impact on the decision relating to the release of P. centaureae on spotted knapweed in North America, especially if safflower is found to be infected by these rust variants already present in North America.

CHAPTER IX. GENERAL CONCLUSION

The European survey for and collection of autoecious Puccinia rusts attacking spotted knapweed in its native range resulted in the discovery of isolates highly virulent on North American populations of spotted knapweed. One isolate from Romania was selected for further studies. The urediniospores morphology of this rust isolate has been studied with light and electron microscopy, and is typical of Puccinia centaureae DC.

Extensive host specificity studies of this P. centaureae isolate were conducted at the quarantine facility of Macdonald College, in order to determine the safety of this potential biocontrol agent of spotted knapweed. Under controlled environment conditions, the host range of P. centaureae is restricted but not confined to spotted knapweed. P. centaureae was able to infect 25 species of Centaurea and species from three other related genera of Cynareae never reported to harbor this rust in Europe. It is possible that this extended host range represents a controlled environment phenomenon and thus may not represent the true field range. Such a phenomenon has frequently occurred in host range studies of plant pathogens and also insects (Dunn 1978;

Peschken and Johnson 1979; Yarwood 1959). The major concern of these results is the indication that safflower, Carthamus tinctorius, may be a potential host of P. centaureae. However, a high level of host resistance to P. centaureae was observed among many safflower cultivars. This resistance appears to be more prominent than the resistance reported on these cultivars against safflower rust, P. carthami. It is interesting to note that the foliage phase of safflower rust does not cause any economic yield loss in the commercial production areas of safflower in the United States (Urie et al. 1968; Zimmer and Jensen 1970; Zimmer and Urie 1968a). The threat that P. centaureae could pose on safflower appears to be negligeable since the degree of infection on safflower at the seedling and heading stages with P. centaureae has not caused significant stress to the safflower. Resistance would also be available from many safflower cultivars and also wild safflower, Carthamus lanatus. It is obvious that further studies are needed before the approval for the release of this rust in North America. Such studies should be focused on the following areas:

1. By field experimentation, determine if P. centaureae can survive on safflower and cause economic yield loss. These experiments would have to be conducted in semi-quarantine facilities in North America or performed in Europe.

2. Study in more detail the life cycle of P. centaureae and

determine if a seedling phase, as reported for P. carthami, also exists for the spotted knapweed rust since this phase of the safflower rust has been reported to cause reduction in safflower stands through seed-borne infection by teliospores of P. carthami.

3. Since both P. centaureae and P. carthami can infect the same host under controlled environment conditions, there is the possibility of crossing the two rusts and studying the pathogenecity of these new recombinants on safflower and thus evaluating the risk of hybridization.

P. centaureae represents a promising candidate for the biological control of spotted knapweed. This leaf rust would fill an open niche on spotted knapweed plants that has not yet been occupied by the insects released on this weed in North America. If approved for release in North America and if a virulent aggressive isolate is established on spotted knapweed, the rust will perhaps add stress to the host to reduce its competitive ability and survival capabilities.

CHAPTER X. SUMMARY

During the evaluation of rust fungi for the biological control of spotted knapweed, the following findings were made:

1. The foreign survey in Eastern and Central Europe resulted in the collection of 106 rust specimens on spotted knapweed from 30 different sites.

2. A total of 48 rust collections representing 21 European sites were found to be virulent on seedlings of North American spotted knapweed. One of these collections, RM-05-b, was the most virulent and promising rust collection for the biological control of spotted knapweed.

3. The host range of the isolate of Puccinia centaureae (RM-05-b) was found to be broader than expected under controlled environment conditions. P. centaureae was able to infect 25 of the 52 species of Centaurea tested; Carthamus tinctorius, Cnicus benedictus, and Amberboa moschata were also infected in these tests. Many species tested were highly resistant to the rust isolate. This

extended host range may not represent the true field host range.

4. Seedlings of fifteen safflower cultivars inoculated with urediniospores of P. centaureae were resistant to rust infection. Six cultivars showed a hypersensitive response on the first pair of true leaves. Cultivars Oleic Leed and 14-5 were the only susceptible cultivars.

5. Mature plant resistance to P. centaureae was found in five safflower cultivars. Inoculation at the seedling stage and subsequent re-inoculation at the heading stage produced infection only on the lower leaves of safflower plants. Spotted knapweed was fully susceptible at both seedling and heading stages.

6. Three spotted knapweed rust collections RM-05-b, OS-07-g, and OS-11-d did not differ in their virulence to five safflower cultivars. These collections may represent the same variant of P. centaureae.

7. The examination of urediniospore morphology revealed that 96 spotted knapweed rust collections were typical of P. centaureae and one collection, OS-15-b, was typical of P. jaceae. Differences in hilum surface ultrastuctures were observed between these two species and P. carthami.

APPENDIX

Appendix 1. Pathogenecity of European rust collections on North American populations of spotted knapweed (Centaurea maculosa Lam.)

Rust collection	Host population inoculated	Number of plants inoculated	Number of plants per Infection Type [a]					
			0	0;	1	2	3	4
FC-01-a	SK-8	2				1	1	
	SK-11	2	2					
	SK-15	2	2					
	SK-16	2	2					
	SK-17	8	5			3		
FC-01-b	SK-17	6	5		1			
FC-05-a	SK-11	2	2					
GR-01-a	SK-2	1	1					
	SK-15	1	1					
HG-01-b	SK-4	1				1		
	SK-7	2	2					
	SK-11	2	1				1	
	SK-17	3	3					
HG-01-c	SK-7	2				1		1
	SK-11	4	4					
	SK-15	4			1	3		
	SK-17	12	2			1	8	1

Appendix 1. (Continued)

Rust collection	Host population inoculated	Number of plants inoculated	Number of plants per Infection Type[a]					
			0	0;	1	2	3	4
HG-01-e	SK-16	4	2					2
	SK-17	2	1			1		
HG-02-a	SK-5	1	1					
	SK-11	2	1			1		
	SK-17	3	2			1		
OS-01-a	SK-1	3	1		1	1		
	SK-17	6	5				1	
OS-01-c	SK-15	2	1			1		
	SK-16	4	3					1
	SK-17	2	1			1		
OS-01-d	SK-16	3	2			1		
OS-01-e	SK-17	3	2					1
OS-01-f	SK-11	4	3			1		
OS-01-i	SK-17	3	3					
OS-02-b	SK-1	2	2					
	SK-17	1	1					
OS-02-d	SK-1	1				1		
	SK-16	2	2					

Appendix 1. (Continued)

Rust collection	Host population inoculated	Number of plants inoculated	Number of plants per Infection Type[a]					
			0	0;	1	2	3	4
OS-02-e	SK-1	2	2					
OS-02-h	SK-11	4	3				1	
	SK-17	3	2				1	
OS-03-a	SK-1	3	3					
	SK-11	2	2					
OS-03-b	SK-8	3	3					
	SK-17	3	3					
OS-04-a	SK-17	2	2					
OS-05-a	SK-11	8	3			1	2	2
	SK-17	16	8			3	6	
OS-05-c	SK-1	1					1	
OS-05-d	SK-17	4	3				1	
OS-05-e	SK-11	1						1
	SK-17	7	5				2	
OS-05-f	SK-1	4	2		1	1		
	SK-11	10	8			1	1	
	SK-17	10	7			2		1

Appendix 1. (Continued)

Rust collection	Host population inoculated	Number of plants inoculated	Number of plants per Infection Type[a]					
			0	0;	1	2	3	4
OS-05-g	SK-17	5	5					
OS-05-h	SK-17	6	3			1	2	
OS-06-a	SK-17	5	3			1	1	
OS-07-a	SK-8	2	1				1	
	SK-17	4	4					
	SK-19	1	1					
OS-07-c	SK-11	3	2				1	
	SK-17	10	8				2	
OS-07-d	SK-17	6	5			1		
OS-07-e	SK-4	2	2					
	SK-8	3	2			1		
	SK-17	4	3			1		
OS-07-f	SK-16	3	2			1		
	SK-17	5	3				2	
OS-07-g	SK-1	4	2			1	1	
	SK-10	1	1					
	SK-11	2	2					
	SK-17	16	5			1	10	

Appendix 1. (Continued)

Rust collection	Host population inoculated	Number of plants inoculated	Number of plants per Infection Type[a]					
			0	0;	1	2	3	4
OS-09-a	SK-5	1	1					
	SK-7	1	1					
	SK-12	4	3				1	
	SK-15	2	2					
	SK-17	14	11			1	2	
OS-09-b	SK-11	4	2		2			
	SK-16	4	3				1	
	SK-17	8	6		1		1	
OS-09-c	SK-11	6	5				1	
	SK-16	3	1				2	
	SK-17	9	6			1	2	
OS-11-a	SK-17	4	4					
OS-11-d	SK-11	3				2	1	
	SK-14	1				2	1	
	SK-17	13	1		4	7	1	
OS-12-a	SK-17	2	2					
OS-12-b	SK-17	3	3					

Appendix 1. (Continued)

Rust collection	Host population inoculated	Number of plants inoculated	Number of plants per Infection Type[a]					
			0	0;	1	2	3	4
OS-12-d	SK-17	22	5			5	12	
OS-13-a	SK-11	3	2				1	
	SK-17	3	3					
OS-14-a	SK-4	4	4					
	SK-17	29	1			9	19	
OS-14-b	SK-16	3	3					
	SK-17	13	7		1	3	2	
OS-14-c	SK-14	4	1			3		
	SK-15	4					4	
	SK-16	4	3				1	
	SK-17	14	8			1	5	
OS-14-e	SK-11	8	3	1	2	1	1	
	SK-15	4	2				2	
	SK-16	8	6				2	
	SK-17	5	2		1	2		
OS-14-f	SK-17	7	4				3	

Appendix 1. (Continued)

Rust collection	Host population inoculated	Number of plants inoculated	Number of plants per Infection Type[a]					
			0	0;	1	2	3	4
OS-15-a	SK-11	2					1	1
	SK-15	10	1			3	6	
	SK-17	18	2			13	3	
OS-15-b	SK-17	5	4				1	
OS-15-c	SK-17	5				5		
OS-15-e	SK-11	3	3					
	SK-17	3	3					
OS-16-a	SK-1	2	1			1		
	SK-17	15	10			1	4	
OS-16-c	SK-17	4	4					
OS-17-a	SK-7	2	1				1	
	SK-15	2	2					
	SK-17	2	2					
OS-17-b	SK-17	6	5				1	
OS-17-c	SK-7	1				1		
	SK-17	5	4			1		

Appendix 1. (Continued)

Rust collection	Host population inoculated	Number of plants inoculated	Number of plants per Infection Type[a]					
			0	0;	1	2	3	4
OS-17-e	SK-9	1					1	
	SK-17	8	5			2		1
OS-17-f	SK-11	3	3					
	SK-16	3	1			1	1	
	SK-17	5	5					
OS-18-a	SK-17	6	6					
RM-04-a	SK-11	2						2
RM-05-b	SK-4	1						1
	SK-8	2					1	1
	SK-17	14	3				5	6

[a] Rating System:

0 Immune: No uredinia nor other symptoms.

0; Nearly immune: No uredinia, but hypersensitive spots present.

1 Very resistant: Uredinia minute, surrounded by distinct necrotic area.

2 Moderately resistant: Uredinia small to medium, usually in green islands surrounded by a chlorotic or necrotic border.

3 Moderately susceptible: Uredinia medium in size, no necrosis but chlorotic areas may be present. Coalescence of uredinia is infrequent.

4 Very susceptible: Uredinia large, and often coalescing. No necrosis, but chlorosis may be present.

REFERENCES

REFERENCES

Allan, R. E., L. H. Pandry, and V. A. Vogel. 1966. Inheritance of seedling and adult reaction of wheat to stripe rust. Crop Sci. 6: 242-245.

Andres, L. A. 1981. Conflicting interests and the biological control of weeds. Pages 11-20 in E. S. Del Fosse (ed.) Proc. V Int. Symp. Biol. Contr. Weeds. Brisbane, Australia. July 1980. 649 p.

Andres, L. A., C. J. Davis, P. Harris, and A. J. Wapshere. 1976. Biological control of weeds. Pages 481-499 in P. B. Huffaker and P. S. Messenger (eds.) Theory and practice of biological control. Academic Press. N.Y.

Anonymus. 1968. The biological control of weeds. pages 86-119 in Principles of plant and animal pest control. Vol.2. Weed Control. National Academy of Sciences. Washington, D.C. 469 p.

Arthur, J. C. 1962. Manual of the rusts in the United States and Canada. Hafner Publ. Co. New York. 438 p.

Ashri, A. 1971. Evaluation of the world collection of safflower, Carthamus tinctorius L. I. Reaction to several diseases and associations with morphological characters in Israel. Crop Sci. 11: 253-257.

Backsay, L. 1958. Annales d' Histoire Naturelle du Musée National de Hongrie, S. N. 50: 121-125.

Batra, W. T. 1982. Biological control in agroecosystems. Science 28: 134-139.

Beldie, A. 1977. Flora Romaniei: I. Determinator ilustrat al Plantelor vasculare. Bucuresti. 209 p.

Braun-Blanquet, J. 1932. Plant sociology: The study of plant community. McGraw Hill. New York. 439 p.

Brown, M. F. and H. G. Brotzman. 1976. Procedures for obtaining sectional views of fungal fructifications by scanning electron microscopy. Can. J. Microbiol. 22: 1252-1257.

Browning, J. A. 1974. Relevance of knowledge about natural ecosystems to development of pest management programs for agro-ecosystems. Proc. Amer. Phytopathol. Soc. 1: 191-199.

Browning, J. A. 1981. The agro-ecosystem -- natural ecosystem dichotomy and its impact on phytopathological concepts. Pages 159-171 in J. M. Thresh (ed.) Pests, pathogens and vegetations. Pitman Adv. Publ. Progr. 517 p.

Browning, J. A., M. D. Simons, and E. Torres. 1977. Managing host genes: Epidemiologic and genetic concepts. Pages 191-212 in J. G. Horsfall and E. B. Cowling (eds.) Plant Disease: an advanced treatise. vol. II. Academic Press. New York. 436 p.

Burdon, J. J. 1978. Mechanisms of disease control in heterogenous plant populations -- an ecologist's view. Pages 193-200 in P. R. Scott and A. Brainbridge (eds.) Plant disease epidemiology. Blackwell. Oxford.

Burdon, J .J., R. H. Groves and J. M. Cullen. 1981. The impact of biological control on the distribution and abundance of Chondrilla juncea in south-eastern Australia. J. Appl. Ecol. 18: 957-966.

Burdon, J. J. and R. C. Shattock. 1980. Disease in plant communities. Appl. Biol. 5: 145-219.

Cabrera, A. L. 1977. Mutisieae -- systematic review. Pages 1039-1066 in V. H. Heywood, J. B. Harborne and B. L. Turner (eds.) The Biology and chemistry of the Compositae. vol. II. Academic Press. New York. 560 p.

Canada Weed Commitee. 1969. Common and botanical names of weeds in Canada. Can. Dep. Agric. Publ. 1397. 67 p.

Cavallito, C. J. and J. H. Baily. 1949. An antibacterial principle from Centaurea maculosa. J. Bacteriol. 57: 207-212.

Conners, I. L. 1943. The rusts of safflower. Phytopathology 33: 789-796.

Coulson, J. 1967. Epidemiology of infectious plant diseases. Lecture notes. Plant Science Dept. McGill University. 543 p.

Cronquist, A. 1955. Phylogeny and taxonomy of the Compositae. Amer. Midl. Natural. 53: 478-511.

Cronquist, A. 1977. The Compositae revisited. Brittonia 29: 137-153.

Cummins, G. B. 1978. Rust fungi on legumes and composites in North America. Univ. Arizona Press. Tucson, Arizona. 424 p.

Dittrich, M. 1977. Cynarae -- systematic review. Pages 999-1015 in V. H. Heywood, J. B. Harborne, and B. L. Turner (eds.) The biology and chemistry of the Compositae. vol. 2. Academic Press. New York. 560 p.

Dostal, J. 1976. Centaurea L. pages 254-301 in T. G. Tutin, V.H. Heywood, N. A. Burgess, D. M. Moore, D. H. Valentine, S. M. Walter, and D. A. Webb (eds.) Flora Europaea. vol. 4. Plantaginaceae to Compositae (and Rubiaceae). Cambridge Univ. Press. 505 p.

Dunn, P. H. 1978. Shortcomings in the classic tests of candidate insects for the biological control of weeds. pages 51-56 in T. E. Freeman (ed.) Proc. IV Int. Symp. Biol. Contr. Weeds. Gainesville, Florida. August 1976. 299 p.

Dyck, P. L., D. J. Samborski, and R. G. Anderson. 1966. Inheritance of adult-plant leaf rust resistance derived from common wheat varieties Exchange and Frontana. Can. J. Genet. Cytol. 8: 665-671.

Ellinghoe, A. H. 1981. Changing concepts in host-pathogen genetics. Ann. Rev. Phytopathol. 19: 125-143.

Emge, R.G, J. J. Melching, and C. H. Kingsolver. 1981. Epidemiology of Puccinia chondrillina, a rust pathogen for the biological control of rush skeleton weed in United States. Phytopathology 21: 839-843.

Expert Committee on weeds. 1983. Report of the research appraisal and planning committee. Western Canada Sect. Vancouver, B. C.

Ferron, M. et R. Cayouette. 1964. Noms des mauvaises herbes du Québec. Minist. Agric. et Colonization du Québec. Publ. 288. 113 p.

Fletcher, E. F. 1913. Further wool-waste plants at Westford, Massachussetts. Rhodora 15: 192.

Fletcher, R. A. and A. J. Renney. 1963. A growth inhibitor found in Centaurea spp. Can. J. Plant Sci. 43: 475-481.

Flor, H. H. 1955. Host parasite interaction in flax -- its genetics and other implications. Phytopathology 45: 680-685.

Fragoso, R. G. 1924. Flora Iberica, Uredales. Tomo. I: Genero Puccinia. Museo Nacional de Ciancas Naturales, Madrid. 416p.

Frankton, C. and G. A. Mulligan. 1970. Weeds of Canada. Can. Dep. Agric. Publ. 948. 217 p.

Freeman, T. E., R. Charudattan, and K. E. Conway. 1978. Status of the use of plant pathogens in the biological control of weeds. Pages 201-206 in T. E. Freeman (ed.) Proc. IV Int. Symp. Biol. Contr. Weeds. Gainesville, Florida. August 1976. 298 p.

Gaumann, E. A. 1952. The Fungi. Hafner Publ. New York. 420 p.

_____. 1959. Die Rostpilze Mitteleuropas. Buchler & Co. Bern. 1407 p.

Goeden, R. D. 1971. Insect ecology of silver leaf nightshade. Weed Sci. 19: 45-51.

_____. 1974. Comparative survey of phytophagous insect faunas of Italian thistle, Carduus pycnocephalus, in southern california and southern Europe relative to the biological weed control. Environ. Entomol. 3: 464-474.

_____. 1977. Biological control of weeds. Pages 43-47 in B. Truelove (ed.) Research methods in Weed Science. South. Weed Soc. Auburn Print. Inc. Auburn. Alabama.

Groh, H. 1944. Canadian weed survey. 2nd Ann. Rep. Can. Dep. Agric. 74 p.

Guinochet, M. 1957. Contribution à l' étude caryologique du genre Centaurea L. sens. lat. Bull. Soc. Hist. Nat. Afr. Nord 48: 282-300.

Guinochet, M. et J. Foissac. 1962. Sur les caryotypes de quelques espèces du genre Centaurea L. et leur signification taxonomique. Rev. Cytol. Biol. Veg. 25: 373-378.

Guyot, A. L. 1967. Les rouilles des Centaurées. Uredineana 6: 49-161.

Harlan, J. R. 1976. Diseases as a factor in plant evolution. Ann. Rev. Phytopathol. 14: 31-51.

Harris, P. 1971. Current approaches to biological control of weeds. Pages 67-76 in Biological Control Programmes Against Insects and Weeds in Canada 1959-1968. Commonw. Inst. Biol. Control Techn. Commun. No. 4.

_____. 1980a. Effects of Urophora affinis Frfld. and U. quadrifasciata (Meig.) (Diptera: Tephritidae) on Centaurea diffusa Lam. and C. maculosa Lam. (Compositae). Z. Angew. Entomol. 90: 190-201.

_____. 1980b. Establishment of Urophora affinis Frfld. and U. quadrifasciata (Meig.) (Diptera: Tephritidae) in Canada for the biological control of diffuse and spotted knapweed. Z. Angew. Entomol. 89: 504-514.

Harris, P. and R. Cranston. 1979. An economic evaluation of control methods for diffuse and spotted knapweed in western Canada. Can. J. Plant Sci. 59: 375-382.

Harris, P. and J. H. Myers. 1984. Centaurea diffusa Lam. and C. maculosa Lam., diffuse and spotted knapweed (Compositae). in Biological Control Programmes against Insects and Weeds in Canada 1969-1981. Tech. Commun. Commonw. Inst. Biol. Control.

Harris, P. and G. L. Piper. 1970. Ragweed (Ambrosia spp.; Compositae), its North American insects and the possibilities for its biological control. Commonw. Inst. Biol. Control Tech. Bull. 43: 117-140.

Harris, P. and H. Zwölfer. 1968. Screening of phytophagous insects for biological control of weeds. Can. Entomol. 100: 295-303.

Hasan, S. 1972. Specificity and host specialization of Puccinia chondrillina. Ann. Appl. Biol. 72: 257-263.

_____. 1974a. First introduction of a rust fungus for the biological control of skeletonweed. Phytopathology 64: 253-254.

_____. 1974b. Xanthium rust as a possible biological control agent of Bathurst and noogoora burrs in Australia. Misc. Publ. Commonw. Inst. Biol. Control. 8: 137-140.

_____. 1980. Plant pathogens and biological control of weeds. Rev. Plant Pathol. 59: 349-356.

_____. 1981. A new strain of the rust fungus Puccinia chondrillina for biological control of skeletonweed in Australia. Ann. Appl. Biol. 99: 119-124.

Hasler, A. 1908. Beitrage zur kenntnis der Crepis-und Centaurea -- Puccinien von Typus der Puccinia hieracii. Centr. Bakt. II. 21: 510-512.

Hayek, A. 1931. Prodromus florae peninsulae Balcanicae. Reptert. Spec. Nov. Reg. Veg. Beih. vol. 30 (2). Berlin-Dahlen.

Heath, M. C. 1982. Host defense mechanisms against infection by rust fungi. Pages 223-245 in K. J. Scott and A. K. Chakravorty (eds.) The Rust Fungi. Academic Press. New York. 287 p.

Hegi, G. 1912. III. Flora von Mitteleuropa. 6: 969-973.

Hooker, A. L. 1967. The genetics and expression of resistance in plants to rusts of the genus Puccinia. Ann. Rev. Plant Pathol. 5: 163-186.

Hubbard, W. A. 1970. Knapweed control. Canadex 641. Can. Dep. Agric.

Huffaker, C. B. 1957. Fundamentals of biological control of weeds. Hilgardia 27: 101-157.

_____. 1973. Fundamentals of biological weed control. Pages 631-649 in P. DeBach (ed.) Biological control of insect pests and weeds. Halsted Press. New York.

Ialongo, M. T. and P. E. Boldt. 1977. Ricerche Preliminari sulla specificita di Puccinia centaureae DC. su Centaurea sphaerocephala L. Annali dell' Instituto Sperimental per la Patologia Vegetale V (1976-1977); 3-7.

Inman, R. E. 1971. A primary evaluation of Rumex rust as a biological agent of curly dock. Phytopatholgy 61: 102-107.

Jacky, E. 1899. Die Compositen -- bewohnenden Puccinien vom Typus der Puccinia hieracii und deren Spezialisierung. 19. Puccinia centaureae Mart. Z. Planzenkr. 9: 330-334.

Jones, B. J. 1977. Vernonieae -- systematic review. Pages 501-521 in V. H. Heywood, J. B. Harborne, and B. L. Turner (eds.) The biology and chemistry of the Compositae. Vol. 1. Academic Press. New York. 619 p.

Kiraly, Z. 1980. Defense triggered by the invador: Hypersensitivity. Pages 201-224 in J. G. Horsfall and E. B. Cowling (eds.) Plant Disease: an advanced treatise. Academic Press. New York. 534 p.

Klokov, M. B., D. I. Sosnovskii, N. N. Tselev, and C. K. Cherepanov. 1963. Centaurea. Flora U.S.S.R. XXVII, 361-535. Moscow & Leningrad. 653 p.

Knott, D. R. 1972. Using race-specific resistance to manage the evolution of plant pathogens. J. Environ. Qual. 1: 227-231.

Leppik, E. E. 1965. Some viewpoints on the phylogeny of rust fungi. V. Evolution of biological specializaton. Mycologia 57: 7-22.

_____. 1967. Some viewpoints on the phylogeny of rust fungi. VI. Biogenic radiation. Mycologia 59: 568-579.

_____. 1970. Gene centers of plants as sources of disease resistance. Ann. Rev. Plant Pathol. 8: 323-343.

Maddox, D. M. 1979. The knapweeds: Their economics and biological control in the western states, U.S.A. Rangelands 1: 139-141.

_____. 1982. Biological control of diffuse knapweed (Centaurea diffusa) and spotted knapweed (C. maculosa). Weed Sci. 30: 76-82.

Marshall, D. R., J. J. Burdon, and A. H. D. Brown. 1981. Optimal sampling strategies for the biological control of weeds. Pages 103-111 in E. S. Del Fosse (ed.) Proc. V Int. Symp. Biol. Contr. Weeds. Brisbane, Australia. July 1980. 649 p.

McCain, A. H. 1963. Inheritance of pathogenicity in safflower rust (Puccinia carthami). Phytopathology 53: 184-186.

Monya, M., M. Sabaw, and G. Racz. 1968. Die antibiotische wirking von Auszügen aus Centaurea-Arten [English summary]. Planta Med. 16: 58-62.

Moore, R. J. 1969. How weedy thistles came to Canada. Greenhouse-Garden-Glass. 8: 1-2.

_____. 1972. Distribution of native and introduced knapweeds (Centaurea) in Canada and the United States. Rhodora 74: 331-346.

Moore, R. J. and C. Frankton. 1954. Cytotaxonomy of three species of Centaurea adventive in Canada. Can. J. Bot. 32: 182-186.

_____. 1974. The thistles of Canada. Can. Dep. Agric. Monogr. 10. 111 p.

Moseman, J. G. 1971. Co-evolution of host resistance and pathogen virulence. pages P50-456 in Barley Genet., Proc. Int. Barley Genet. Symp. 1969.

Muller, H., D. Schroeder, and A. Gassmann. 1982. Investigations on Agapeta zoegana Haw. (Lep.: Cochylidae), a possible biocontrol agent of spotted knapweed, Centaurea maculosa Lam. (Compositae) in Canada. Commonw. Inst. Biol. Contr. Report. Delemont, Switzerland. 21 p.

Myers, J. H. and S. Ockenden. 1977. Metzenaria paucipunctella as a potential biological control agent of spotted knapweed in Westwold, British Columbia. Pages 132-134 in Proc. Knapweed Symp. October 1977. Kamploops, B.C. 292 p.

Nelson, R. R. 1978. Genetics of horizontal resistance to plant diseases. Ann. Rev. Phytopathol. 16: 359-378.

_____. 1979. The evolution of parasitic fitness. Pages 23-46 in J. G. Horsfall and E. B. Cowling (eds.) Plant disease: an advanced treatise. Academic Press. New York. 466 p.

Oehrens, E. 1977. Biological control of blackberry through the introduction of rust, Phragmidium violaceum, in Chile. F.A.O. Plant Prot. Bull. 25: 26-28.

Peschken, D. P. and G. R. Johnson. 1979. Host specificity and suitability of Lema cyanella (Coleoptera: Chrysomelidae), a candidate for the biological control of Canada thistle (Cirsium arvense). Can. Entomol. 111: 1059-1068.

Petersen, R. H. 1974. The rust fungus life cycle. Bot. Rev. 40: 453-511.

Popular, C. 1978. Changes in host susceptibility with time. Pages 239-262 in J. G. Horsfall and E. E. Cowling (eds.) Plant Disease: an advanced treatise. Vol. 2. Academic Press. New York. 436 p.

Prasad, R. 1947. The rust of safflower Puccinia carthami (H.) Corda. Curr. Sci. 16: 292

Reed, C. F. and R. O. Hughes. 1970. Selected weeds of the United States. U. S. Dep. Agric. Handb. 366. 463 p.

Robinson, R. A. 1976. Plant Pathosystems. Springer-Verlag. New York. 184 p.

Room, P. M. 1981. Biogeography, apparancy, and exploration for biological control agents in exotic ranges of weeds. Pages 113-124 in E. S. Del Fosse (ed.) Proc. V Int. Symp. Biol. Contr. Weeds. Brisbane, Australia. 649 p.

Rousseau, C. 1968. Histoire, habitat et distribution de 220 plantes introduites au Québec. Nat. Can. 95: 49-169.

Rouy, G. et E. G. Camus. 1901. Flore de France. Paris.

Sackston, W. E. 1953. Foot and root infection by safflower rust in Manitoba. Plant Disease Reptr. 37: 522-523.

Salinska, M. R. Czapik, and M. Piotrowicz. 1959. Further studies in chromosome numbers of Polish Angiosperm. Acta. Soc. Bot. Pol. 28: 487-529.

Samborski, D. J. and P. L. Dyck. 1966. Inheritance of virulence in wheat leaf rust on standard differential wheat varieties. Can. J. Genet. Cytol. 10: 24-32.

_____. 1976. Inheritance of virulence in Puccinia recondita on six backcross lines of wheat with single genes for resistance to leaf rust. Can. J. Bot. 54: 1666-1671.

Sands, D. P. A. and K. L. S. Harley. 1981. The importance of geographic variation in agents selected for the biological control of weeds. Pages 81-90 in E. S. Del Fosse (ed.) Proc. V Int. Symp. Biol. Contr. Weeds. Brisbane, Australia. July 1980. 649 p.

Savile, D. B. O. 1944. Safflower. pages 28-29 in I. L. Conners and D. B. O. Savile (compilers). 23rd Ann. Report Can. Plant Dis. Surv. - 1943. 122 p.

_____. 1970a. Some Eurasian Puccinia species attacking Cardueae. Can. J. Bot. 48: 1553-1566.

_____. 1970b. Autoecious Puccinia species attacking Cardueae in North America. Can. J. Bot. 48: 1567-1584.

_____. 1971. Coevolution of the rust fungi and their hosts. Quat. Rev. Biol. 46: 211-218.

_____. 1973. A variety of Puccinia centaureae on Centaurea diffusa. Can. J. Bot. 51: 1077-1078.

_____. 1979. Fungi as aids in higher plant classification. Bot. Rev. 45: 377-503.

Schroeder, D. 1977. Biotic agents attacking diffuse and spotted knapweed in Europe and their prospective suitability for biological control in North America. Pages 108-131 in Proc. Knapweed Symp. October 1977. Kamloops, British Columbia. B. C. Min. Agric. Victoria. 293 p.

_____. 1983. Biological control of weed. pages 41-78 in W. W. Fletcher (ed.) Recent advances in weed Research. Commonw. Agric. Bur.

Schuster, M. L. 1956. Investigations on the foot and root phase of safflower rust. Phytopathology 46: 591-595.

Schuster, M. L. and D. W. Christiansen. 1952. A foot and root disease caused by Puccinia carthami Cda. Phytopathology 42: 211-212.

Segal, J., G. Fischbeck, and I. Wahl. 1980. How plant populations defend themselves in natural ecosystems. Pages 75-102 in J. G. Horsfall and E. B. Cowling (eds.) Plant Disease: an advanced treatise. Vol. 5. Academic Press. New York. 534 p.

Shaner, G. and R. L. Powelson. 1971. Epidemiology of stripe rust of wheat, 1961-1968. Oreg. Agric. Exp. Stn. Tech. Bull. 117.

Spears, B. M., S. T. Rose, and W. S. Belles. 1980. Effect of canopy cover, seedling depth, and soil moisture on emergence of Centaurea maculosa and C. diffusa. Weed Res. 20: 87-90.

Stakman, E. C. , D. M. Stewart, and W. Q. Loerging. 1962. Identification of physiologic races of Puccinia graminis var. tritici. U. S. Dep. Agric. A. R. S. Bull. E617. (Revised 1962). 52 p.

Storey, J. M. and N. L. Anderson. 1978. Release and establishment of Urophora affinis (Diptera: Tephritidae) on spotted knapweed in western Montana. Environ. Entomol. 7: 445-448.

Sydow, P. and H. Sydow. 1904. Monographia Uredinearum, 1,1. Bortraegen Lipsiae. 972 p.

Templeton, G. E. and R. J. Smith. 1977. Managing weeds with pathogens. Pages 167-175 in J. G. Horsfall and E. B. Cowling (eds.) Plant Disease: an advanced treatise. Vol. 2. Academic Press. New York. 436 p.

Templeton, G. E. and D. O. Te Beest. 1979. Biological weed control with mycoherbicides. Ann. Rev. Phytopathol. 17: 301-310.

Thomas, C. A. 1955. A new race of safflower rust. Plant Dis. Reptr. 39: 652-653.

_____. 1958. Reactions of safflower varieties to regional collections of Puccinia carthami. Plant Dis. Reptr. 42: 1089-1090.

Traquair, J. E. and E. G. Kokko. 1983. Urediniospore morphology of Puccinia species attacking Cardueae. Can. J. Bot. 61: 2047-2051.

Trotter, A. 1908. Flora Italica Cryptogame, Urdinales. Rocca S. Casciano. 519 p.

Tutin, T. G., V. H. Heywood, N. A. Burgess, D. M. Moore, D. H. Valentine, S. M. Walter, and D. A. Webb. 1976. Flora Europaea. Vol. 4. Plantaginaceae to Compositae (and Rubiaceae). Cambridge Univ. Press. 505 p.

Urie, A. L., L. N. Leininger, and D. E. Zimmer. 1968. Effects of degree and time of defoliation on yield and related attributes of safflower. Crop Sci. 8: 747-750.

Van der Planck, J. E. 1968. Disease resistance in plants. Academic Press. New York. 206 p.

_____. 1975. Principles of plant infection. Academic Press. New York. 216 p.

_____. 1982. Host-plant interaction in plant disease. Academic Press. New York. 270 p.

Wagner, H. 1977. Cynareae -- chemical review. Pages 1017-1038 in V. H. Heywood, J. B. Harborne, and B. L. Turner (eds.) The biology and chemistry of the Compositae. Vol. 2. Academic Press. New York. 560 p.

Wapshere, A. J. 1974a. Host specificity of phytophagous organisms and the evolutionary centres of plant genera and sub-genera. Entomophaga 19: 301-309.

_____. 1974b. A strategy for evaluating the safety of organisms for biological weed control. Ann. Appl. Biol. 77: 201-211.

_____. 1975. A protocol for programmes for biological control of weeds. P.A.N.S. 21: 295-303.

_____. 1978. Effectiveness: A comparaison of prediction and results during the biological control of Chondrilla. Pages 124-127 in T.E. Freeman (ed.) Proc. IV Int. Symp. Biol. Contr. Weeds. Gainsville, Florida. August 1976. 298 p.

_____. 1981a. Recent thoughts on exploration and discovery for biological control of weeds. Pages 75-79 in E. S. Del Fosse (ed.) Proc. V Int. Symp. Biol. Contr. Weeds. Brisbane, Australia. July 1980. 649 p.

_____. 1981b. The biological control of Paterson's curse, Echium plantagineum: Northern Hemisphere studies. Pages 599-602 in E. S. Del Fosse (ed.) Proc. V Int. Symp. Biol. Contr. Weeds. Brisbane, Australia. July 1980. 649 p.

_____. 1983. Problems in the use of plant biochemistry for establishing the safety of biological control agents for weeds; the Chondrilla and Echium / Heliotropium cases. Entomophaga 28: 287-294.

Watson, A. K. 1977. Biology of spotted knapweed. pages 20-25 in Proc. Knapweed Symp. Kamloops, British Columbia. October 1977. Min. Agric., Victoria. 292 p.

Watson, A. K. and I. Alkhoury. 1981. Response of safflower cultivars to Puccinia jaceae collected from diffuse knapweed in Eastern Europe. Pages 301-305 in E. S. Del Fosse (ed.) Proc. V Int. Symp. Biol. Contr. Weeds. Brisbane, Australia. July 1980. 649 p.

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

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

Watson, L. 1972. Smuts on grasses: some general implications of the incidence of Ustilaginales on the genera of Gramineae. Quat. Rev. Biol. 47: 46-62.

Weiss, E. A. 1971. Castor, Sesame and Safflower. Barnes & Nobles Inc. New York. 865 p.

Wilson, C. L. 1969. Use of plant pathogens in weed control. Ann. Rev. Phytopath. 7: 411-433.

Wilson, M. and D.M. Henderson. 1966. British rust fungi. Cambridge University Press. London. 384 p.

Winder, J. A. and K. S. Harley. 1978. Exploration for organisms for biological control of weeds. Pages 35-38 in T. E. Freeman (ed.) Proc. IV Int. Symp. Biol. Contr. Weeds. Gainesville, Florida. August 1976. 298p.

Yarwood, C. E. 1959. Predisposition. Pages 521-562 in J. G. Horsfall and A. E. Dimond (eds.). Plant Pathology; an advanced treatise. Academic Press. New York. 674 p.

Zadocks, J. C. 1961. Yelloow rust on wheat. Studies on epidemiology and physiologic specialization. Tijdschr. Plantez. 76: 69-256.

_____. 1972. Methodology of epidemiological research. Ann. Rev. Phytopath. 10: 253-276.

Zhukovsky, P. M. 1959. Vzaimootnosheniia mezhdu khozainom i gribnym parazitom na ikh rodine i vue ie [Interrelation between host and parasite in their origin and beyond it]. Vestn. S-h. Nanki, Moscow. 4: 25-34. (In russian, with English summary).

Zimmer, D. E. 1962. Hypocotyl reaction to rust infection as a measure of resistance in safflower. Phytopathology 52: 1177-1180.

_____. 1963. Response of safflower varieties and lines to cultures of Puccinia carthami. Plant Dis. Reptr. 47: 486-488.

_____. 1965. Rust infection and histological response of susceptible and resistant safflower. Phytopathology 55: 296-301.

Zimmer, D. E. and J. J. Jensen. 1970. Seedling rust and yield of safflower. Plant Dis. Reptr. 54: 364-367.

Zimmer, D. E. and A. L. Urie. 1967. Significance of seedling rust resistance of safflower and its relationship to foliage rust resistance. Phytopathology 57: 773-776.

_____. 1968a. Influence of foliage-rust on yield, test weight, and oil percentage of safflower. Plant Dis. Reptr. 52: 876-878.

_____. 1968b. Inheritance of rust resistance in crosses between cultivated safflower, Carthamus tinctorius, and wild safflower, C. oxycantha. Phytopathology 58: 1340-1342.

_____. 1969. Inheritance of rust resistance in safflower. Crop Sci. 9: 491-494.

Zimmer, D. E., P. Schaelling, and A. L. Urie. 1968. The nature and inheritance of seedling rust resistance on Nebraska 115 safflower. Phytopathology 58: 1451-1456.

Zwolfer, H. 1965. Preliminary list of phytophagous insects attacking wild Cynareae (Compositae) species in Europe. Commonw. Inst. Biol. Control Tech. Bull. 6: 81-154.

Zwolfer, H. 1965. Current investigations on phytophagous insects associated with thistles and knapweeds. pages 63-67 in F. J. Simmonds (ed.) Proc. First Int. Symp. Biol. Contr. Weeds. Delemont, Switzerland. March 1969.

Zwolfer, H. and P. Harris. 1971. Host specificity determination of insects for biological control of weeds. Ann. Rev. Entomol. 16: 159-178.