Pathogenicity of *Agrobacterium* Species from the Noxious Rangeland Weeds *Euphorbia esula* and *Centaurea repens*

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ABSTRACT

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Disease surveys were made of Russian knapweed (*Centaurea repens*) and leafy spurge (*Euphorbia esula*), two noxious weeds that severely infest large areas of rangelands in the northern Great Plains. Strains of *Agrobacterium tumefaciens* were isolated from Russian knapweed exhibiting crown galls in New Mexico in 1991. Strains pathogenic to one or more of *Helianthus annuus, Lycopersicon esculentum, Nicotiana tabacum, Datura stramonium*, or Russian knapweed and known strains of biovars 1 and 2 of *A. tumefaciens* and *A. vitis* were inoculated on Russian knapweed and two other knapweed species, diffuse (*C. diffusa*) and spotted (*C. maculosa*). Some strains from New Mexico were strongly pathogenic to diffuse knapweed, causing rapidly developing galls that typically girdled, stunted, and caused death of the host. The biovar 1 strains were pathogenic to all three knapweed species, strains of biovar 2 were pathogenic to diffuse and spotted knapweed, and the single *A. vitis* strain was pathogenic only to diffuse knapweed, forming small galls. Stunted and chlorotic plants of leafy spurge with crown galls, collected in Glacier County, Montana, and plants with root galls collected in eastern North Dakota were infected with *A. tumefaciens*, which was identified as biovar 1. Leafy spurge plants exhibiting galls on roots collected in North Dakota were infected with strains identified as biovars 1 and 2. Host ranges among nine pathogenic strains from Russian knapweed, leafy spurge, and known strains representing biovars 1 and 2 of *A. tumefaciens* and *A. vitis* varied greatly, with six of the nine strains being pathogenic to no more than one additional species besides the original host. These findings indicate that *A. tumefaciens* may be effective as a biological control of these important rangeland weeds and especially of diffuse knapweed.

The noxious weed species leafy spurge (*Euphorbia esula* L.) and Russian, spotted, and diffuse knapweed (*Centaurea repens* L. [*Acroptilon repens* (L.) DC.], *C. maculosa* Lam., and *C. diffusa* Lam., respectively) cause widespread and serious infestations of rangelands in several Rocky Mountain and northern Great Plains states and Prairie Provinces of North America. The area infested by the three principal knapweeds was estimated to be approximately 4.8 million hectares (8) in 1991. The total area infested by leafy spurge was estimated in 1991 to exceed 1 million hectares, with annual economic losses estimated to be $110 million in five Great Plains states (2). These weeds are detrimental to wildlife habitats, aesthetics, and biodiversity (8,17) within infested areas. Furthermore, the weeds are gastrically irritating or toxic to livestock (8,11,15). Infestations occur on private property and such public lands as national parks and wildlife refuges.

The use of herbicides for control of these aggressive, persistent weeds is generally not considered to be economically feasible; thus, means for their biological control are being investigated. The aggressive and tenacious nature of these weeds is largely because of vigorous and extensive root systems (16,17). Logically, then, biological control strategies should be concentrated principally on the root systems.

Stunted and chlorotic plants of leafy spurge with crown galls were collected in Glacier County, Montana, in 1991, and plants of leafy spurge collected in western North Dakota in 1991 were later observed to develop root galls in the greenhouse. Russian knapweed plants exhibiting stunting associated with crown gall were collected in 1991 in New Mexico. Crown gall is among the few soilborne diseases that have been found domestically on Russian knapweed and leafy spurge (4). Therefore, this study was conducted to investigate the potential of *Agrobacterium tumefaciens* as a biological control agent of the knapweeds and leafy spurge. *A. tumefaciens* is also among the most common pathogens found on *E. esula* in Europe, which is part of its native range (*unpublished*). There the weed is apparently under natural control and is an inconspicuous member of roadside flora. The objectives of the present study were to investigate the host range and virulence of the native strains of *A. tumefaciens* from leafy spurge and Russian knapweed and to assess their potential as biological control agents.

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MATERIALS AND METHODS

Isolations from Russian knapweed and leafy spurge. Plants of Russian knapweed exhibiting crown galls were collected in the summer of 1991 in New Mexico. Leafy spurge exhibiting crown galls was collected in Glacier County, Montana. Another sample consisted of apparently healthy roots of leafy spurge collected in eastern North Dakota and later discovered in the greenhouse to have crown galls. The galled plants were thoroughly washed under running tap water and blotted dry with a sterile paper towel, and gall tissue was excised from stems with a sterile scalpel. Galls were diced and pieces of gall tissue were placed in test tubes of a sterile phosphate buffer (pH 7), and the tubes were agitated on a vortex mixer and incubated overnight at 4 C. The supernatant was streaked onto plates of potato-dextrose agar (PDA), King's medium B (KB), Roy/Sasser medium (RS) (18), and, in the case of the galled roots from North Dakota, also on medium 2E (3). Plates of PDA and KB were incubated at 28 C for 4–5 days, and typical white, glistening, convex (12) colonies of characteristic *Agrobacterium* spp. were selected from PDA plates for pathogenicity tests. Plates of RS were incubated for 6–10 days, which allowed the growth of colonies of *A. tumefaciens* biovar 1 and *A. vitis*, as determined in preliminary studies. Colonies that grew on RS were selected and streaked onto plates of PDA. Plates of medium 2E were incubated for 1 wk at 28 C, and single colonies were selected and streaked onto plates of PDA.

Bacterial storage and inoculum production. To assess for purity and to produce inoculum for pathogenicity tests, 400 strains suspected to be *Agrobacterium* spp. were streaked on PDA. Cultures were grown at 28 C. Purified cultures of putative *Agrobacterium* spp. were streaked onto PDA in test tubes and stored at 4 C. Cryotubes of 2.0-ml capacity filled with 1.5 ml of nutrient broth containing 15% glycerol were also inoculated with pure cultures and stored at −80 C or −20 C.

Pathogenicity tests. The plants were grown in the greenhouse at 20–28 C and watered uniformly at 3-day intervals. Seeds of the various plant species to be inoculated were planted in individual 10.2-cm-diameter pots in a steamed greenhouse soil mix composed of

approximately 33% each of sphagnum peat, sand, and Bozeman silt loam (1:1:1, v/v), pH 6.6. Three to four weeks after planting, the plants were thinned to three per pot. A sterile dissecting needle was used to collect inoculum from colonies growing on culture plates. Plants were inoculated by puncturing stems at the ground line with the needle holding a drop of the fluid matrix containing bacterial cells. Strains from Russian knapweed and leafy spurge were used to inoculate one or more of the following species: sunflower (*Helianthus annuus* L.), tobacco (*Nicotiana tabacum* L.), tomato (*Lycopersicon esculentum* Mill.), and *Datura stramonium* L. Three plants each of 3-wk-old plants were inoculated. Pathogenicity tests were repeated at least once. The inoculated plants were incubated at 20–28 C in the greenhouse and assessed for gall formation after 6 wk. Control plant stems were inoculated similarly with *A. radiobacter* strain A136 or D45 or punctured with a sterile needle.

Inoculation of knapweed species. Bacterial strains that were pathogenic to one or more of the test plant species described above were used to inoculate 24-wk-old Russian knapweed plants propagated from crowns collected in the field and 6-wk-old Russian knapweed seedlings. In order to determine whether the strains possessed any host specificity or differential virulence to the three principal noxious rangeland knapweed species, strains pathogenic to Russian knapweed or to some of the other test plant species were used to inoculate 6-mo-old plants of Russian, diffuse, and spotted knapweed (Table 1). Three plants of each knapweed species were inoculated. Additionally, each of the three knapweed species was inoculated with *Agrobacterium* strains representative of *A. tumefaciens* biovars 1 and 2 and *A. vitis*, one *A. tumefaciens* strain from Montana, and two strains of unknown origin from a culture collection at Montana State University (Table 1). Controls consisted of plants inoculated with two nonpathogenic strains of *Agrobacterium* spp. from galls of Russian knapweed. Plants were incubated as described above. Inoculated plants were assessed for disease severity on a 0–5 rating scale in which 0 = no gall formation; 1 = gall ≤1 cm in diameter, visible in >21 days; 2 = gall 1.1–2 cm in diameter, visible in <21 days; 3 = gall 2.1–3 cm in diameter, visible in <21 days; 4 = gall 3.1–4 cm in diameter, visible in <21 days; and 5 = gall >4 cm in diameter or plant girdled and dead or dying within 31 days. The experiment was repeated twice. Data from the three experiments were pooled for an analysis of variance ($P = 0.05$), and the Waller-Duncan exact Bayesian *k*-ratio LSD rule was used to separate means. The 6-wk-old seedlings were inoculated with the puncture method described above and assessed for relative gall size to determine whether the physiological age of the host species might affect results obtained with older plants. The experiment using seedlings was repeated once.

Pathogenicity of strains from leafy spurge. Strains of *Agrobacterium* spp. that caused gall formation on one or more of the test plant species were tested on leafy spurge. First, 6-mo-old roots of leafy spurge obtained from Sidney, Montana, were sterilized by soaking them in 0.5% sodium hypochlorite for 1 hr, followed by rinsing for 1 hr in running tap water. The plants were left in the water overnight to dissipate residual NaOCl. Roots were blotted to remove excess water and stored in plastic bags at 4 C for 5 days. The crowns of spurge plants were inoculated with the puncture method described above and planted in 15-cm-diameter plastic pots containing pasteurized soil mix. Controls were plants with stems either inoculated with *A. radiobacter* strain D45 or punctured with a sterile needle. Three plants were inoculated with each strain. Plants were harvested 6 wk after inoculation and assessed for the formation of galls. The experiment was repeated twice.

Host range tests. Strains representative of the several pathogenic strains from the two weed hosts, i.e., strains of biovar 1 from leafy spurge from Montana, strains of biovars 1 and 2 from leafy spurge roots from North Dakota, and strains of biovar 1 from Russian knapweed, were used to inoculate 10 cultivated species for an assessment of host range. Inoculation methods, replications, and experimental design were identical to those described above. Species inoculated were artichoke (*Cynara scolymus* L. 'Green Globe'), sweet corn (*Zea mays* L. 'Golden Bantam'), flax (*Linum usitatissimum* L.), mung bean (*Vigna radiata* (L.) R. Wilcz. 'Berken'), peanut (*Arachis hypogaea* L. 'Virginia Jumbo'), snap bean (*Phaseolus vulgaris* L. 'Blue Lake 274'), soybean (*Glycine max* (L.) Merr.), sugar beet (*Beta vulgaris* L.), sunflower cv. D131, and zinnia (*Zinnia violacea* Cav. 'Zenith Mixed'). The negative controls were plants inoculated with *A. radiobacter* strain D-45. Positive controls were sets of three plants of one or more of the host species on which individual strains had originally caused galls. Three plants per strain were inoculated, harvested 6 wk later, and assessed for the formation of galls. The experiment was repeated twice.

Characterization of pathogenic and nonpathogenic *Agrobacterium* strains. Strains were identified to biovar using the Biolog Version 3.5 series of prepared biochemical tests (Biolog, Hayward, CA) and confirmed on the basis of reactions of the strains to the 3-ketolactose and litmus milk tests, production of acid from erythritol or malonate, tolerance to sodium chloride, and growth at 35 C on either medium 1A, 2E (3), or RS (18). All tests were repeated at least once.

RESULTS

Pathogenicity tests and identification of pathogenic strains. Approximately 160 strains isolated from galls of Russian knapweed were tested for pathogenicity on one or more of four test plant species, followed by tests on Russian knapweed. Six pathogenic strains were determined to be *A. tumefaciens* biovar 1.

Of 240 strains isolated from crown and root galls on leafy spurge from two different locations, 35 were determined to be pathogenic to at least one of the four test species. All of the 17 pathogenic strains isolated from galls on leafy spurge from Glacier County, Montana, were biovar 1 and all were pathogenic to leafy

Table 1. Origin of *Agrobacterium* spp. strains described in the present study

Strain	Original host	Identification	Source
AG83A	Russian knapweed	*A. tumefaciens* biovar 1	A. J. Caesar
AG83C	Russian knapweed	*A. tumefaciens* biovar 1	A. J. Caesar
AG135	Russian knapweed	*A. tumefaciens* biovar 1	A. J. Caesar
AG135A	Russian knapweed	*A. tumefaciens* biovar 1	A. J. Caesar
AG138	Russian knapweed	*A. tumefaciens* biovar 1	A. J. Caesar
AG139	Russian knapweed	*A. tumefaciens* biovar 1	A. J. Caesar
North Dakota strains			
91-25 No. 25	Leafy spurge	*A. tumefaciens* biovar 1	A. J. Caesar
91-25 No. 23	Leafy spurge	*A. tumefaciens* biovar 1	A. J. Caesar
91-25 No. 7	Leafy spurge	*A. tumefaciens* biovar 2	A. J. Caesar
Montana strains			
91-30 No. 21	Leafy spurge	*A. tumefaciens* biovar 1	A. J. Caesar
91-30 No. 38	Leafy spurge	*A. tumefaciens* biovar 1	A. J. Caesar
Control strains			
A723	Unknown	*A. tumefaciens* biovar 1	T. McCoy
AT	Unknown	*A. tumefaciens* biovar 1	D. C. Sands
D-60	Russian knapweed	*A. radiobacter*	A. J. Caesar
D-45	Russian knapweed	*A. radiobacter*	A. J. Caesar
15955	Unknown	*A. tumefaciens* biovar 1	L. W. Moore
K27	Unknown	*A. tumefaciens* biovar 2	L. W. Moore
CG48	Grape	*A. vitis*	L. W. Moore

spurge. Of the 17 pathogenic strains isolated from root galls of leafy spurge from North Dakota, eight were biovar 1 and nine were biovar 2; three strains (two of biovar 1 and one of biovar 2) were pathogenic to leafy spurge.

Comparative aggressiveness of strains from Russian knapweed to three knapweed species. When the pathogenic strains from Russian knapweed were tested on Russian, spotted, and diffuse knapweed, the latter species was the most susceptible to the various strains of *A. tumefaciens* representing three biovars. Disease ratings obtained beginning 6 wk after inoculation indicated the severity of disease on diffuse knapweed caused by strains of biovar 1 (Table 2), which rapidly formed large galls (Fig. 1). These galls girdled and killed diffuse knapweed within 6–12 wk. All plants of diffuse knapweed inoculated with biovars 1 and 2 died within 4 mo (Fig. 2). Although Russian and spotted knapweed were less susceptible, individual strains, e.g., AG83A (Table 2), were highly virulent to Russian knapweed. Significant differences in virulence also occurred among strains on individual knapweed species, and some strains, e.g., AG83C (Table 2), were apparently more virulent to some knapweed species than to others. When seedlings of the various knapweeds were inoculated with the set of strains referred to above, the relative gall sizes and disease progress were similar to those observed with older, mature plants (*data not shown*).

Host range tests. The host ranges varied among representative strains from Russian knapweed, leafy spurge, *A. tumefaciens* biovars 1 and 2, and *A. vitis*. All strains were nonpathogenic to corn, artichoke, and peanut (Table 3). Three strains—one each from Russian knapweed, leafy spurge, and strain 15955 of biovar 1—were pathogenic to more than a single species, causing gall formation on five, three, and three species, respectively. All three of these strains were pathogenic to flax and zinnia. All other strains were pathogenic to no more than one of the 10 test species. Strain CG48 was highly pathogenic to sugar beet, and two other strains of biovar 1 were also pathogenic.

Table 2. Disease ratings of Russian, diffuse, and spotted knapweed inoculated with strains of *Agrobacterium* spp. from Russian knapweed and other sources, representing three biovars

Strain	Biovar	Disease ratings[z]		
		Russian	Diffuse	Spotted
AG83A	1	4.0 a	4.2 ab	4.0 a
15955	1	3.3 ab	4.8 a	4.2 a
AG135	1	3.3 ab	3.8 abc	2.9 ab
CG48 (*A. vitis*)	...	3.0 ab	1.7 cd	0.0 d
AG135A	1	2.5 abc	4.8 a	2.0 abcd
K27	2	1.2 bc	3.7 abc	3.2 ab
AT	1	0.5 c	2.0 bcd	0.5 cd
AG83C	1	0.4 c	3.3 abc	1.3 bcd
D-60 (*A. radiobacter*)	...	0.0 c	0.0 d	0.0 d
A723	1	0.0 c	4.0 abc	3.7 a
AG139	1	0.0 c	0.0 d	0.0 d
AG138	1	0.0 c	2.3 bcd	2.3 abcd

[z] Inoculated plants were assessed for disease severity on a rating scale in which 0 = no gall formation; 1 = gall ≤1 cm in diameter, visible >21 days; 2 = gall 1.1–2 cm in diameter, visible in <21 days; 3 = gall 2.1–3 cm in diameter, visible in <21 days; 4 = gall 3.1–4 cm in diameter, visible in <21 days; and 5 = gall >4 cm in diameter or plant girdled and dead or dying within 31 days. In each column, means followed by the same letter are significantly different by the Waller-Duncan exact Bayesian *k*-ratio LSD rule. Each mean is based on ratings of a total of nine plants.

Fig. 1. Crown gall on diffuse knapweed (*Centaurea diffusa*) 3 wk after inoculation with *Agrobacterium tumefaciens* strain AG83A. (Approximately 2×)

DISCUSSION

Strains of *A. tumefaciens* from Russian knapweed are highly virulent to diffuse knapweed and are of potential value for the biological control of diffuse knapweed. To our knowledge, this is the first report of crown gall diseases of leafy spurge and Russian knapweed. The severe disease on diffuse knapweed observed in greenhouse tests seems unusual, since infection with *A. tumefaciens* is normally not lethal and yield losses are not great (L. Moore, *personal communication*) but can be measurable and statistically significant (13,19). A survey of references to major works on *Agrobacterium* spp. indicates that most research on crown gall diseases has been concerned with woody perennials (9), and thus the susceptibility of herbaceous plants may have been overlooked.

The isolation of strains of *A. tumefaciens* representing two biovars from a single gall or host population agrees with established findings concerning the biology of crown gall disease of other plant species (1,14). The occurrence of galls on leafy spurge inhabited by biovars 1 and 2 at one location and by a single biovar at another location may indicate that the former instance is a coincidence, that there is some genetic variation among leafy spurge populations in different regions, or that the finding is a result of the detection limits of the procedures used.

The relatively narrow host ranges of the various strains that were determined in the present study are supported by previous studies that have shown that single strains may be narrow in host range (1,10). Furthermore, the results of the present study and previous ones (1,10) indicate the possibility that screening several strains may yield ones with narrow host ranges and high virulence. The finding herein that strains of biovar 2 from leafy spurge affected no more than the original host, compared with the range of one to five hosts observed with strains of biovar 1 from leafy spurge, is of value. Both biovars caused disease on leafy spurge, but biovar 1 did so with greater frequency. However, because of their narrower host range, the strains of biovar 2 might be of greater utility as biological control agents of leafy spurge. But there are precautions to be noted even with the use of narrow host range strains. The occurrence of large, rapidly formed galls on sugar beet caused by a strain of *A. vitis* indicates that the value for biocontrol of a strain of narrow host

Table 3. Reaction of nine cultivated species to *Agrobacterium* strains from Russian knapweed and leafy spurge[y]

Host Strain[z]	Species								
	Artichoke	Flax	Mung bean	Peanut	Snap bean	Sugar beet	Soybean	Sunflower	Zinnia
Russian knapweed									
AG83A	−	+	−	−	−	+	−	+	+
D-45	−	−	−	−	−	−	−	−	−
Leafy spurge									
91-25 No. 25	−	−	−	−	−	−	−	−	−
91-25 No. 23	−	−	−	−	−	−	−	−	−
91-25 No. 7	−	−	−	−	−	−	−	−	−
91-30 No. 21	−	+	−	−	−	+	−	+	+
91-30 No. 38	−	−	−	−	−	−	−	+	−
Unknown									
15955	−	+	+	−	−	−	−	+	+
K27	−	−	−	−	−	−	−	−	−
Grape									
CG48	−	−	−	−	−	+	−	−	−

[y]Three plants of each species per strain were inoculated at the ground line by puncturing stems with a dissecting needle covered with bacterial cells. Plants were harvested 6 wk later and assessed for formation of galls. Positive controls were sets of three plants of one or more of the host species on which individual strains had originally caused galls. The experiment was repeated twice.
[z]The 91-25 strains were isolated from galls that developed in the greenhouse on roots of leafy spurge collected in western North Dakota. The 91-30 strains were isolated from crown galls on leafy spurge collected in Glacier County, Montana.

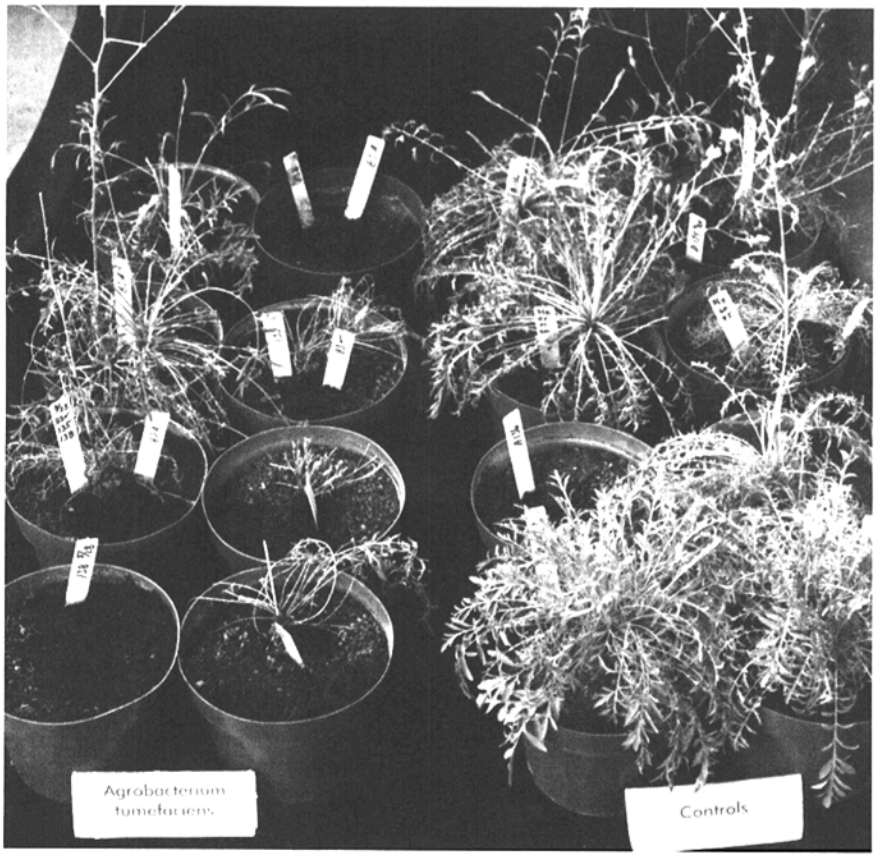

Fig. 2. Greenhouse-grown diffuse knapweed (*Centaurea diffusa*) plants 3 mo after inoculation with various strains of *Agrobacterium tumefaciens* biovar 1 originally isolated from Russian knapweed (*Centaurea repens*). Most inoculated plants had died and the rest died within 1 mo. Plants were 2 yr old when inoculated; results were similar in inoculated seedlings and 6-mo-old plants.

range may be mitigated if one of those few hosts is severely affected. However, *A. vitis* has not been an economic problem on sugar beet and, furthermore, has only been recovered naturally from grape, yet it can infect many hosts in the greenhouse (T. J. Burr, *personal communication*).

Most research on the biological control of weeds using plant pathogens is concerned with annual weeds in crops (7), and not with herbaceous perennial weeds (most of this latter category are aquatic or plant-parasitic) that are aggressive through extensive and prolific root growth. Previous successes with mycoherbicides on annual broadleaf weeds are therefore generally not instructive in regard to the biological control of perennials with plant pathogens. New strategies of biological control must be developed that employ methods appropriate to the biology of the weeds and that can interface with insect biological control agents currently being deployed against leafy spurge and the knapweed species. *Pythium* and *Fusarium* spp. were isolated from crown galls (*unpublished*). This finding is of interest and may indicate the value of synergism, whether opportunistic or manipulated, to effective biocontrol. Biocontrol with *A. tumefaciens* could be enhanced by applications of suitable strains of other soilborne pathogens as galls are formed on inoculated plants. Such strains have been discovered and described (4–6), and others are the subject of research currently under way. The importance of secondary infection leading to significant pathogenicity, with crown gall as the starting point, has been noted previously (19). The requirement of a wound for infection by *A. tumefaciens* is perhaps an advantage and opens the possibility of a manipulated synergism with insect biological control agents that are specific to one of the target weeds noted above.

LITERATURE CITED

1. Anderson, A. R., and Moore, L. W. 1979. Host specificity in the genus *Agrobacterium*. Phytopathology 69:320-323.
2. Bangsund, D. A. 1991. Economic impact of leafy spurge in Montana, South Dakota, and Wyoming. N.D. State Univ. Agric. Econ. Rep. 275.
3. Brisbane, P. G., and Kerr, A. 1983. Selective media for all three biovars of *Agrobacterium*. J. Appl. Bacteriol. 54:425-431.
4. Caesar, A. J. 1994. Comparative virulence of strains of *Rhizoctonia* spp. on leafy spurge (*Euphorbia esula*) and disease reactions of cultivated plants in the greenhouse. Plant Dis. 78:183-186.
5. Caesar, A. J., Rees, N. E., Spencer, N. R., and

Quimby, P. C. 1993. Diseases of leafy spurge in the Northern Great Plains. Pages 2-37-40 in: Leafy Spurge Symposium. R. A. Masters, S. J. Nissen, and G. Friisoe, eds. Great Plains Agric. Counc. Publ. 144.
6. Caesar, A. J., Rees, N. E., Spencer, N. R., and Quimby, P. C., Jr. 1993. Characterization of *Rhizoctonia* spp. causing disease of leafy spurge in the Northern Plains. Plant Dis. 77:681-684.
7. Charudattan, R. 1991. The mycoherbicide approach with plant pathogens. Pages 24-57 in: Microbial Control of Weeds. D. O. TeBeest, ed. Chapman & Hall, New York.
8. Lacey, J. R., and Olson, B. E. 1991. Environmental and economic impacts of noxious range weeds. Pages 5-29 in: Noxious Range Weeds. L. F. James, J. O. Evans, M. H. Ralphs, and R. D. Childs, eds. Westview Press, Boulder, CO.
9. Lippincott, J. A., Lippincott, B. B., and Starr, M. P. 1983. The genus *Agrobacterium*. Pages 842-855 in: The Prokaryotes. M. P. Starr, ed. Springer-Verlag, New York.
10. Loper, J. E. , and Kado, C. I. 1979. Host range conferred by the virulence-specifying plasmid of *Agrobacterium tumefaciens*. J. Bacteriol. 139:591-596.
11. Lorenz, R. J., and Dewy, S. A. 1988. Noxious weeds that are poisonous. Pages 309-336 in: The Ecology and Economic Impact of Poisonous Plants on Livestock Production. L. F. James, M. H. Ralphs, and D. B. Nielson, eds. Westview Press, Boulder, CO.
12. Moore, L. W., Kado, C. I., and Bouzar, H. 1988. II. Gram-negative bacteria. A. *Agrobacterium*. Pages 16-36 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. 2nd ed. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN.
13. Moore, L. W., and Tingey, D. T. 1976. Effects of temperature, plant age, and infection site on the severity of crown gall disease in radish. Phytopathology 66:1328-1333.
14. Nesme, X., Michel, M.-F., and Digat, B. 1987. Population heterogeneity of *Agrobacterium tumefaciens* in galls of *Populus* L. from a single nursery. Appl. Environ. Microbiol. 53:655-659.
15. Panter, K. E. 1991. Neurotoxicity of the knapweeds in horses. Pages 316-324 in: Noxious Range Weeds. L. F. James, J. O. Evans, M. H. Ralphs, and R. D. Child, eds. Westview Press, Boulder, CO.
16. Rees, N. E., and Spencer, N. R. 1991. Biological control of leafy spurge. Pages 182-192 in: Noxious Range Weeds. L. F. James, J. O. Evans, M. H. Ralphs, and R. D. Child, eds. Westview Press, Boulder, CO.
17. Roché, B. F., and Roché, C. D. 1991. Identification, distribution, ecology, and economics of *Centaurea* species. Pages 274-291 in: Noxious Range Weeds. L. F. James, J. O. Evans, M. H. Ralphs, and R. D. Child, eds. Westview Press, Boulder, CO.
18. Roy, M., and Sasser, M. 1983. A medium selective for *Agrobacterium tumefaciens* biotype 3. (Abstr.) Phytopathology 73:810.
19. Schroth, M. N., McCain, A. H., Foott, J. H., and Huisman, O. C. 1988. Reduction in yield and vigor of grapevine caused by crown gall disease. Plant Dis. 72:241-246.