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Biocontrol of Common St. Johnswort (*Hypericum perforatum*) with *Chrysolina hyperici* and a Host-Specific *Colletotrichum gloeosporioides*[1]

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Abstract: Common St. Johnswort is widespread in eastern Canada but it seldom constitutes a serious weed problem. A demographic study conducted in 1993 and 1994 at four typical undisturbed sites indicated that 36 to 96% of established St. Johnswort shoots died during the growing season. Mortality was always associated with infection by a host-specific *Colletotrichum gloeosporioides.* The leaf-feeding beetle *Chrysolina hyperici* occurred at all sites and caused maximum midsummer defoliation of 27% in 1993 and 51% in 1994. Healthy plants readily recovered from defoliation during pupation of the fourth instar of the insect in June and following adult estivation in August. Although widespread, *C. hyperici* populations appear transient and alone do not cause sustained feeding pressure resulting in weed control. When *Chrysolina* larvae and adults were collected at six field sites and placed on healthy seedlings under controlled conditions, up to 36% of the plants became infected with *C. gloeosporioides.* Scanning electron micrographs commonly showed *Colletotrichum* conidia among the setae on legs, tarsal pads, and antennae of adults and larvae. In a series of three experiments conducted under controlled conditions in which *Chrysolina* larvae and adults were placed on healthy plants after feeding on diseased ones, the incidence of infection ranged from 63 to 100%. Hence, under favorable conditions *Chrysolina* adults may selectively transmit the pathogen in the field. This study demonstrated the potential of enhancing biological control of weeds by insects with the integration of an effective, host-specific pathogen.

Nomenclature: Common St. Johnswort, *Hypericum perforatum* L. #[3] HYPPE; *Chrysolina hyperici* (Forester) (Coleoptera: Chrysomelidea); *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.

Additional index words: Classical biological control, disease, demography, herbivory, integrated control, *Aphis chloris.*

Abbreviations: *C.g.-hypericum, Colletotrichum gloeosporioides* f. sp. *hypericum*; PDA, potato dextrose agar.

INTRODUCTION

Common St. Johnswort is native to the Mediterranean region but has been widely disseminated. It is a toxic and aggressive weed of rangelands, open forests, and other uncultivated habitats (Campbell and Delfosse 1984; Crompton et al. 1988). Successful biological control programs, based principally on two introduced leaf-feeding Chrysomella beetles, *Chrysolina quadrigemina* and *C. hyperici,* were initiated in the 1930s in Australia and subsequently in the western United States and Canada (Campbell and McCaffrey 1991; Clark 1953; Harris et al. 1969; Harris and Maw 1984). Control has consistently been best in open, arid sites where *C. quadrigemina* predominates (Harris and Maw 1984; Shepherd 1985; Williams 1985), and several years of heavy defoliation, coupled with succession of native vegetation, are necessary for control (Campbell and McCaffrey 1991; Williams 1985). *Chrysolina* spp. are less effective on moist, shaded sites where the survival rate of seedlings and defoliated plants is greater (Campbell and McCaffrey 1991; Shepherd 1985). Although other insects have been assessed for St. Johnswort control (Campbell and McCaffrey 1991; Harris et al. 1969; Harris and Maw 1984; Shepherd 1985), other agents are still needed, particularly those effective in moist or shaded habitats (Shepherd 1985; Williams 1985).

Observations on St. Johnswort in Nova Scotia since 1985 have shown that, although widespread, it is usually not a serious problem requiring specific control mea-

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[3] Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds,* Revised 1989. Available from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

sures. *Chrysolina hyperici* was introduced into Nova Scotia in 1969 (Harris and Maw 1984) and is now widely distributed. Populations of the insect are erratic and transient and do not appear to cause sustained feeding pressure resulting in effective control of the weed. Low St. Johnswort densities may be related to the widespread occurrence of an anthracnose disease induced by a *Colletotrichum gloeosporioides* (Crompton et al. 1988; Hildebrand and Jensen 1991). This pathogen is host-specific and highly virulent on St. Johnswort and, when applied as a mycoherbicide, controls all stages of the weed (Hildebrand and Jensen 1991).

Colletotrichum species have been studied extensively as potential mycoherbicides on a number of weed species (Auld and Morin 1995; Quimby and Birdsall 1995; Templeton et al. 1979; Templeton 1992), generally in cultivated annual crops where there is potential for commercial development. Here, inundative applications of conidia are required to induce disease because timely natural epidemics seldom occur under cultivation due, in part, to poor inoculum dissemination and persistence (TeBeest 1982, 1991; Templeton et al. 1979). Rain splash, generally < 0.5 m/event, is the most common mechanism of *Colletotrichum* dispersal within the plant canopy (Yang and TeBeest 1992b), but the sticky spore matrix also facilitates dispersal by fauna (Libby and Ellis 1954; Nemeye et al. 1990; Yang and TeBeest 1992; Yang et al. 1994).

In contrast, *Colletotrichum* species have received little attention in the context of classical biological control (Quimby and Birdsall 1995; Templeton 1992), although the potential has been cited (Templeton et al. 1979). *Colletotrichum*-induced diseases can give significant control of some weeds in uncultivated habitats (Butler 1951; Hildebrand and Jensen 1991; Morris 1982; Wicker 1967) where timing of weed control may be less important and persistence and timely dissemination of inoculum less limiting.

Insects are vectors of some *Colletotrichum*-induced diseases (Libby and Ellis 1954; Nemeye et al. 1990; Yang et al. 1994) and can disseminate conidia between infected and noninfected weed stands (Yang et al. 1994). We conducted two field experiments in 1992 that suggested that *Chrysolina* adults might be transmitting *Colletotrichum gloeosporioides* on St. Johnswort in Nova Scotia (Jensen and Doohan 1994). In an experiment investigating the spread of the pathogen among equidistantly transplanted St. Johnswort plants, the pattern of disease development following an unexpected influx of *Chrysolina* adults was random and was not related to proximity to the central inoculated plant. In another experiment assessing the pathogen as a mycoherbicide in a pasture with a resident *Chrysolina* population, there was little difference in disease levels after 3 mo between treated and control plots. Despite some early-season treatment differences, control of St. Johnswort exceeded 90% after one season on both treated and spatially separated control plots in both experiments. These patterns of disease development could not be accounted for by wind-driven rain splash.

We initiated this study in 1993 to examine the influence of *Colletotrichum gloeosporioides* and *Chrysolina hyperici* on St. Johnswort in Nova Scotia. We (1) conducted a demographic study to determine factors affecting St. Johnswort mortality in undisturbed habitats, (2) examined *C. hyperici* as a vector of *C. gloeosporioides* under field and controlled environment conditions, and (3) examined the potential of using *C. hyperici* contaminated with *C. gloeosporioides* as a biocontrol agent.

METHODS AND MATERIALS

Plant Demography. A demographic study was established in June 1993 at four undisturbed sites near Windsor, NS (45° N; 64°10′ E) to examine factors affecting St. Johnswort mortality. One hundred plants at various stages of growth and showing no obvious anthracnose symptoms were randomly selected and tagged in each of two abandoned pastures (A and B) and along a graveled railroad embankment and a riverbank. These habitats were typical of those where St. Johnswort occurs locally; the weed comprised $< 2\%$ of the ground cover. Disease assessments of topgrowth with lesions or chlorotic or necrotic tissue were made on each tagged plant at 2-wk intervals from mid-June to mid-August and once in mid-September and -October on a ranking scale of 0 (no apparent disease), 1 (1 to 10%), 2 (11 to 25%), 3 (26 to 49%), 4 (50 to 89%), and 5 (90 to 100%). The occurrence of *C. gloeosporioides* was confirmed periodically by incubating surface-sterilized subsamples of diseased stem sections on moist filter paper or on potato dextrose agar (PDA). Total leaf area of each plant lost to herbivory was also ranked on a scale of 0 (no defoliation), 1 (1 to 5%), 2 (6 to 10%), 3 (11 to 25%), 4 (29 to 49%), 5 (50 to 89%), and 6 (90 to 100%). The midpoints of these categories were used for statistical analysis. Because only specialized feeders can consume the toxic foliage of St. Johnswort (Fields et al. 1990), all herbivory was attributed to *Chrysolina.* Defoliation assessments ceased in mid-August when *Chrysolina* adults estivate. The presence of *Chrysolina hyperici* eggs, larvae,

or adults was recorded on each date. Other common insects resident on the weed, e.g., spittlebugs (*Philaenus* spp.) and aphids (*Aphis* spp.), were also recorded. Distance from each tagged plant to the nearest neighboring St. Johnswort plant was measured in June, patch size was estimated in July, and number of seed capsules per plant were recorded in August. Seedlings were not common, but in August 1993, 30 and 100 St. Johnswort seedlings were tagged at pasture A and railroad sites, respectively, and disease levels were recorded as above.

In May 1994, winter survival of each tagged plant was determined, and moribund plants were replaced by new randomly selected plants. Here, moribund refers to completely dead topgrowth but does not preclude new shoots arising later from basal buds on the crown or rhizomes, which sometimes was observed. At the riverbank site, new plants had to be selected due to ice scour of the original site during winter flooding. Assessments commenced in mid-May 1994 and were conducted as in 1993.

The following variables were analyzed by a series of Kruskal–Wallis tests to determine site differences: mean levels of disease and of insect herbivory over the season, mean patch size, and distance of each tagged plant to its nearest neighbor. Means were compared using a multiple comparison procedure (Dunn 1964) when treatment effects were significant ($P < 0.05$). Chi-square analysis was also performed to determine site differences for presence or absence of *C. hyperici* larvae and adults, spittlebugs, aphids, and unidentified leafrollers.

Data were also analyzed separately for each year using a logistic regression model for each of the following binomial responses: (1) healthy vs. diseased plants; (2) of plants that were diseased, those with $< 50\%$ disease vs. those with $> 50\%$; and (3) of plants with $> 50\%$ disease, those that developed symptoms before mid-July vs. after mid-August. The analyses combined data from the four sites. The independent variables were patch size and presence or absence of *Chrysolina* larvae or adults, aphids, and spittlebugs. A second analysis, which excluded the riverbank site, was performed for the plants that survived until May 1994 vs. those that did not. The independent variables were disease incidence, i.e., (1) no disease, (2) $< 50\%$ disease and (3) $> 50\%$ disease developing before mid-July, or (4) disease developing after early August; patch size; and presence or absence of *C. hyperici* larvae or adults, spittlebugs, or aphids. In both sets of analyses, independent variables were entered into the model in a forward stepwise fashion, and only variables that were significant at $P < 0.15$ were included in the final model.

***Chrysolina hyperici* as Vectors.** *General procedures.* In the following experiments, St. Johnswort plants were grown to the appropriate stage in a greenhouse as previously described (Hildebrand and Jensen 1991). The greenhouse was operated with ambient irradiance and photoperiod and with 22 C day and 18 C night set-point temperatures. Three growth stages of plants were used depending on the experiment: (1) young, nonbranching seedlings, 6 to 8 cm tall; (2) advanced seedlings, four to eight procumbent and upright stems up to 25 cm long; and (3) early flowering, six to eight stems up to 35 cm tall. *Chrysolina,* at various stages of growth and development, were field-collected locally and held for several days before the experiments commenced in 45 by 45 by 65 cm screened insect cages containing potted flowering St. Johnswort plants. Insect cages were kept in controlled environment chambers operated at 23 C day and 15 C night temperatures, 14-h photoperiod, and 70% relative humidity. Cages were steam-sterilized before each experiment. All laboratory inoculations were done with an isolate of *Colletotrichum gloeosporioides* originating from a culture (DAOM 195231) deposited at the Canadian National Mycological Collection (Hildebrand and Jensen 1991). Isolates of this pathogen are host-specific, and hereafter all are referred to as *C.g.-hypericum.* Conidia were produced in liquid culture and plants were inoculated by applying an aqueous suspension of 10^6 conidia/ml with 0.1% Tween 80 to run-off as previously described (Hildebrand and Jensen 1991). Plants were then placed for 24 h in mist chambers at 22 C in darkness before being returned to the greenhouse. To produce infected plants for insect transmission studies, inoculated plants with stem lesions were placed for 5 d under a propagation bench mist line to induce formation of acervuli and sporulation. When returned to the greenhouse, plants were subirrigated on capillary matting, and untreated controls were kept in separate greenhouse compartments to minimize cross-contamination.

Experiment 1. An experiment was conducted to determine if *C. hyperici* adults could efficiently transmit *C.g.-hypericum* from diseased to healthy plants under controlled conditions. Two hundred adults were placed in each of two 45 by 45 by 65 cm cages containing either healthy or infected flowering St. Johnswort plants. After 7 d, three beetles were transferred to the base of each of four healthy advanced seedlings contained in smaller (30 by 30 by 30 cm) screened cages. There were six cages with four plants each for each treatment, i.e., adult insects previously exposed to either healthy or infected plants. Six cages of four healthy plants without insect

adults served as controls. Cages were placed in the above growth chambers for 7 d. Beetles were then removed and plants were placed in mist chambers to enhance infection. Plants were then moved to the greenhouse where presence or absence of disease was recorded on each plant after 18 and 30 d. Significance of treatment differences were determined by Fisher's exact test.

Experiment 2. An experiment was conducted in 1994 to determine if *C. hyperici,* collected in the field at various developmental stages, carried and could transmit *C.g.-hypericum* inoculum to healthy plants. *C. hyperici* beetles were collected at three times—early May (first and second instar), late May to early June (third and fourth instar), and early August (adult)—on St. Johnswort growing in six typical habitats, i.e., two roadsides, two pastures, and two lowbush blueberry fields. Head capsule width was used to identify instar stage (Clark 1953). At each time, 50 insects were removed from at least 20 random plants at each site. Each insect was immediately placed on the stem tip of a healthy young St. Johnswort seedling. Plants were misted with sterilized water, covered with a translucent plastic cup, and placed in an outdoor shaded insectary for 2 d. Insects were then removed and plants were placed in the mist chamber for 24 h. They were then removed to the greenhouse. The presence or absence of disease was recorded 20 d later. The following controls were used at each collection time: (1) 15 healthy check seedlings not exposed to insects and (2) 50 seedlings potentially contaminated by a single insect at the appropriate stage that had fed for 2 d on heavily diseased, laboratory-inoculated plants in screened cages and treated as above. The results demonstrated that some infective *Chrysolina* stages were collected from all sites, but numbers of infective individuals were too low to determine life stage or site differences by Chi-square analysis; hence, data are simply presented as percent infected plants.

The presence of *C.g.-hypericum* on *Chrysolina* was also confirmed by examining external body parts for conidia using scanning electron microscopy. Third and fourth instar larvae and adults were collected from laboratory-infected plants and from diseased field plants and were then fixed overnight in 4% glutaraldehyde in 0.07 M sodium phosphate buffer at pH 6.8. Specimens were dehydrated in a series of 10 to 100% acetone washes and dried in a CO_2 critical point drier. Specimens were coated with 15 nm gold/palladium using a Hummer VII sputtering system and viewed with a JEOL JSM-T330A scanning electron microscope.

Experiment 3. An experiment was conducted in July 1994 to examine the effectiveness of *Chrysolina* adults contaminated with *C.g.-hypericum* in transmitting the disease. Experiments were conducted in a lath-house by setting 25 7.5-cm pots of young St. Johnswort seedlings inside 0.5 by 1 m wooden frames then filling the frames to the rim of the pots with moist sand. These frames could be covered by a tight-fitting screen cage to confine the beetles during treatment. Two methods of contaminating field-collected adults were assessed. One was to place adults for 3 d in cages with heavily infected St. Johnswort plants that had been inoculated 10 d previously. The other was to place beetles for 20 to 30 s onto the surface of a sporulating culture of *C.g.-hypericum* on PDA before being transferred to healthy plants. Sporulating cultures were prepared by spraying an aqueous suspension of conidia onto PDA followed by incubation under continuous fluorescent light at 22 C for 5 d. Under these conditions, mycelial growth was suppressed and germinating conidia predominantly produced acervuli. Prior to treatment, 2 ml of sterile water were added to each dish and conidia were released by gentle scraping with a glass rod.

C. hyperici adults were field-collected in late July 1994 and contaminated with *C.g.-hypericum* by the two methods described above. Two beetles were placed near the base of each of the 25 plants/frame and the frames were covered with a screen cage. Controls were similar, except that beetles were kept in cages with healthy plants prior to treatment. Beetles were transferred directly to the seedlings or to sterile PDA and then to the test seedlings. Plants were occasionally moistened by applying mist during the 2-d exposure period. Beetles were removed and plants placed in a mist chamber for 24 h before being returned to the greenhouse. Each treatment and control was replicated twice and the experiment was repeated in 1994.

Due to paucity of beetles, only the PDA contamination treatment was assessed in July 1995 but with the following modifications: plants used were in the early bloom stage, three beetles per plant were used, and plants were not misted despite dry weather during exposure to the beetles. Data from the two years were combined and the numbers of dead, diseased, and healthy plants after 6 wk were ranked as 2, 1, and 0, respectively. These rankings were analyzed by restricted maximum likelihood (Thompson and Welham 1993).

RESULTS AND DISCUSSION

Plant Demography. *Variation among sites.* There were significant differences among the four sites in the fol-

Table 1. Patch size, distance to nearest neighbor, number of seed capsules, and seasonal mean herbivory and disease incidence of St. Johnswort at four sites in 1993 and 1994.[a]

							Seasonal mean			
	Patch size		Distance to neighbor		Seed capsules		Herbivory		Disease	
Demography site	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994
	m^2		cm		no./plant		%			
Pasture A	1.0 b	0.7 a	5 b	34 b	26 b	5 d	18 b	27 b	23 a	42 b
Pasture B	0.3 a	0.5 a	22 c	53 b	29 b	9 a	6 a	26 b	22 a	22 a
Riverbank	7.3 d	16.0 c	7 a	12 a	6 a	6 a	9 a	10 a	64 b	51 b
Railroad embankment	1.9 c	2.8 b	25 c	49 b	132 c	71 b	8 a	21 b	25 a	21 a

[a] Means for each variable followed by the same letter are not significantly different at $P \leq 0.05$ by Dunn's (1964) multiple comparison procedure for nonparametric data.

lowing parameters: patch size, distance between plants, seed production, and seasonal mean percentage of herbivory and disease level (Table 1). Herbivory of young shoot tips and foliage was typical of that of the various life stages of *C. hyperici* described by Fields et al. (1990), and disease symptoms were consistently associated with *C.g.-hypericum.* Similarly, there were significant differences among sites in both years in the percentage of plants hosting *Chrysolina* larvae and adults, spittlebugs, and aphids (Table 2). The aphids were predominately *Aphis chloris,* which had been introduced near the pasture sites in 1991 (M. G. Sampson, personal communication) and now appears well established. An unidentified leafroller was observed at all four sites in 1994. The number of plants hosting adult *Chrysolina* was generally less than those with spittlebugs and aphids.

Temporal variation in appearance of insects and herbivory. Data collection commenced in mid-June 1993 and coincided with the pupation of the fourth instar of *C. hyperici* and rapid vegetative growth of the weed. This resulted in the low levels of defoliation recorded in mid-June of both years (Figure 1). The subsequent increase in defoliation was related to the emergence and active feeding of the adults. Maximum mean defoliation by adults ranged between 7 and 27% in 1993 and between 12 and 51% in 1994. Extensive defoliation occurred only on plants infected with *C.g.-hypericum.* In spring 1994, about 40% defoliation by early instar stages of the insect occurred at two sites, but this had no permanent effect on the plants (Figure 1), nor did later defoliation by the adults, as all healthy plants fully recovered. Adults estivated in August with little evidence of fall feeding. Damage from sucking insects, e.g., spittlebugs and aphids, appeared minimal.

Temporal variation in disease incidence. Initial disease symptoms appeared in early May as lesions on the tip and base of procumbent basal stems that became sunken and dark brown with purple centers. These lesions often girdled the stems. Infected basal stems became wilted and died, and the upright flowering stem became chlorotic and eventually turned a dark, reddish brown. These woody flowering stems often remained standing and produced flowers and seeds despite loss of the basal rosette. Lesions at the top of the plant were less common but could be found on branches, foliage, and the inflorescence. Lesions that girdled the stems of seedlings were fatal.

Table 2. The percentage of St. Johnswort plants at each of the four sites with resident *Chrysolina* larvae and adults, spittlebugs, aphids, and leafrollers in 1993 and 1994.

	Chrysolina								
	Larvae		Adults		Spittlebugs		Aphids		Leafrollers[b]
Demography site	1993[a]	1994	1993	1994	1993	1994	1993	1994	1994
	% of plants								
Pasture A	5	11	23	6	12	21	20	11	7
Pasture B	0	45	12	9	21	37	61	43	32
Riverbank	0	2	17	5	2	5	1	0	9
Railroad embankment	1	42	10	13	23	38	0	38	8
P-value[c]		< 0.01	0.05	NS	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

[a] Cell sizes in 1993 were inadequate for analysis.

[b] This unidentified leafroller was not observed in 1993.

[c] P-values are significance levels of between-site comparisons according to the Chi-square test.

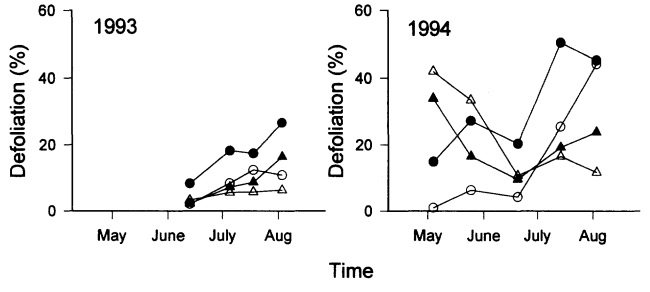

Figure 1. Seasonal trends in defoliation of St. Johnswort attributable to larval and adult stages of *Chrysolina hyperici* in 1993 and 1994 at four sites: pasture A (●), pasture B (△), the riverbank (○), and the railroad embankment (▲). Data points are the means of 100 observations.

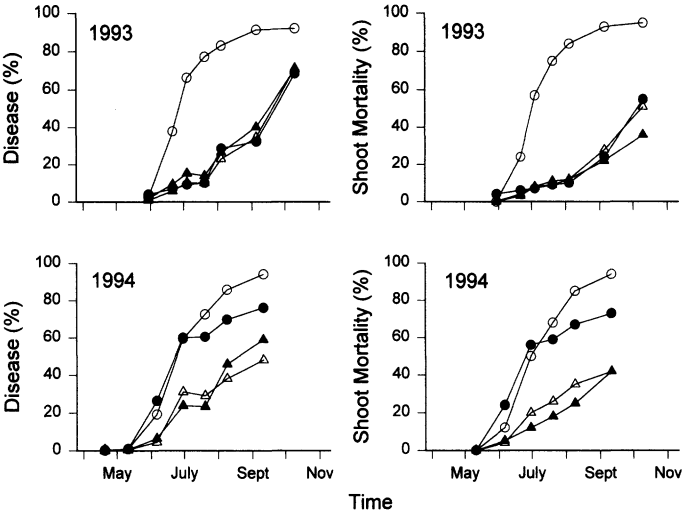

Figure 2. Seasonal trends in disease and shoot mortality attributable to *C.g.-hypericum* in 1993 and 1994 at four sites: pasture A (●), pasture B (△), the riverbank (○), and the railroad embankment (▲). Data points are means of 100 observations.

Symptoms developed rapidly over the summer (Figure 2). By October 1993 and 1994, 96 and 82% of all tagged plants in the study had become infected. Similarly, 94 and 73% of the tagged seedlings also became infected in 1993 and 1994, respectively (data not shown). Disease developed faster at the riverbank site in both years, but in 1994, disease development at one of the pasture sites was similar.

Factors affecting survivorship. Depending on site, shoot mortality (i.e., plant moribundity) by the end of the growing season ranged between 36 and 93% in 1993 and between 42 and 96% in 1994 (Figure 2). The time course of shoot death over the growing season closely reflected the seasonal progression of the disease in both years. Of the initial cohort of plants selected in 1993, those that did not survive until 1994 were associated with greater disease severity, larger patch size, and lower aphid numbers (Table 3).

A categorical model using logistic regression on the combined data from the four sites indicated that disease incidence increased with patch size (Table 4). Plants in larger patches were infected sooner in 1993 but later in 1994. There were no clear and consistent relationships between resident insects, including *Chrysolina,* and plant health, and the majority of comparisons were not significant at $P < 0.15$. In 1993, *Chrysolina* larvae were associated with healthier plants, and in 1994, adults were more common on plants developing symptoms late in the season. In 1994, spittlebugs and aphids were also more common on plants with lower disease ratings and plants that developed > 50% disease late in the season. Although this might suggest that insects tended to choose healthier plants, this was not evident in 1993 when there was a positive relationship between numbers of spittlebugs and disease incidence (Table 4).

***Chrysolina hyperici* as Vectors.** *Experiment 1.* When *Chrysolina* adults were placed on infected St. Johnswort plants under controlled conditions for 2 d and then transferred to healthy ones, 63% of the latter were infected with *C.g.-hypericum* after 30 d (Table 5). In this experiment, 17% of the plants also became infected after contact with beetles that had been kept on healthy plants, suggesting that the field-collected adults were naturally contaminated. Control plants not exposed to beetles remained healthy.

Experiment 2. Some *Chrysolina* larvae and adults collected from all six locations transmitted *C.g.-hypericum* when they were transferred to healthy St. Johnswort seedlings under conditions favorable for infection (Table 6). Each site had at least one developmental stage of the insect that transmitted the disease to healthy plants. After exposure to *Chrysolina* adults collected from one blueberry field, 36% of the seedlings became infected. Among the controls, about 85% of the larvae and 100% of the adults transmitted the disease to the seedlings.

Scanning electron micrographs of various *Chrysolina* stages collected from either field- or laboratory-infected

Table 3. Significant effects of a forward stepwise logistic regression for plants that survived until 1994 and those that did not.[a]

Survival	Disease rating	Aphids	Patch size
	0–5	no.	m^2
Survived to 1994	1.9	77	0.9
Did not survive	2.3	33	1.2
P-value[b]	< 0.01	0.03	0.02

[a] Riverbank site was excluded because of damage to site by ice.

[b] P-values are significance levels for the relationship between survival and the independent variables. Variables that did not affect survival were the presence or absence of *Chrysolina* larvae and adults, spittlebugs.

Table 4. Results of a forward stepwise logistic regression for the following binomial responses: disease status, disease level, and disease development on St. Johnswort at four sites in 1993 and 1994.

	Chrysolina									
	Larvae		Adults		Spittlebugs		Aphids		Patch size	
Binomial response	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994
Disease status										
Healthy	10	21	19	0	24	36	5	30	1.0	1.7
Diseased	1	28	15	10	14	32	21	24	2.7	4.2
(P-value)[a]	(0.02)	NS	NS	NS	NS	NS	(0.05)	NS	(0.04)	(0.03)
Disease level[b]										
< 50%	0	38	7	13	5	48	20	48	0.8	1.5
> 50%	1	24	17	8	16	26	22	15	3.1	5.3
(P-value)	NS	NS	NS	NS	(0.01)	(0.01)	NS	(< 0.01)	(< 0.01)	(0.01)
Disease development[c]										
Early season	0	15	10	2	2	10	0	2	6.4	4.8
Late season	1	26	18	15	18	42	26	31	1.6	5.6
(P-value)	NS	NS	NS	(0.02)	NS	(< 0.01)	NS	(< 0.01)	(< 0.01)	(< 0.01)

[a] P-values are significance levels between the binomial responses. Independent variables significant at $P < 0.15$ were included in the model.

[b] Disease level is the seasonal mean.

[c] Early-season disease development is when > 50% disease was attained before mid-July; late season is when > 50% disease was not attained until after early August.

plants showed that conidia were most commonly found on body parts with setae, e.g., leg joints, tarsi, and antennae (Figure 3).

Experiment 3. Releasing adults contaminated with *C.g.-hypericum* was an effective way of inoculating St. Johnswort under simulated field conditions, despite dry weather during the 2-d exposure to the beetle and relatively inactive late-season adults (Table 7). There was no correlation between feeding injury and frass on the plants and development of disease. Brief exposure of adults on sporulating *C.g.-hypericum* cultures on PDA was more effective than feeding for 2 d on infected plants in transmitting the pathogen to healthy plants, killing or infecting about 80% of the plants compared to 60%.

The demography sites were typical of noncultivated St. Johnswort habitats in Atlantic Canada. In contrast to those in Australia and western North America where St. Johnswort may dominate some sites (Campbell and Delfosse 1984; Harris et al. 1969), in Atlantic Canada it rarely exceeds 5% of the canopy. Pastures A and B were selected because large numbers of adult *Chrysolina* were observed there in the previous year. However, the greatest number of larvae or adults recorded on any date was only 33 adults/100 plants at Pasture A in 1993. These numbers are similar to those recorded earlier by Sampson (1985). Larger numbers of *Chrysolina* were observed on some untagged plants at the sites, and we have occasionally observed numbers of adults with densities of > 100/plant, but overall, this is the exception. However, *Chrysolina* densities may have been higher than those recorded, especially larval densities, because this insect has adopted behavior and partially nocturnal feeding habits that minimize direct exposure to sunlight and photosensitization (Fields et al. 1990). Nevertheless, the maximum mean level of defoliation recorded at any site or life stage was about 50% (Figure 1), and high levels of defoliation occurred only on diseased plants which grew poorly. Reduced feeding activity of late fourth instar larvae and their subsequent pupation resulted, in part, in the decrease in mean St. Johnswort defoliation in June (Figure 1). Healthy plants were observed to recover during pupation and after estivation in late summer. There was no evidence of mass emergence or fall feeding of *Chrysolina* adults, and the fate of these remains unknown.

In Australia, 3 mo of complete defoliation in combination with drought was required to control mature St. Johnswort (Clark 1953). Holloway (1964) reported that 3 yr of heavy feeding controlled the weed in California. In Nova Scotia, St. Johnswort grows actively for 6 to 7

Table 5. Percentage of St. Johnswort plants infected with *C.g.-hypericum* 18 and 30 d after being fed on by *Chrysolina* adults that were previously kept on healthy or infected plants.

	Infected plants	
Treatment	18 d	30 d
	%	
Infected plants	25	63
Healthy plants	4	17
Controls[a]	0	0
P-value[b]	< 0.1	< 0.01

[a] Control plants were not exposed to *Chrysolina* adults and were not included in the analysis.

[b] P-values are probability levels that differences between the two treatments are significant by Fisher's exact test.

Table 6. Percentage of plants that became infected with *C.g.-hypericum* after being fed on by *Chrysolina hyperici* collected from St. Johnswort at six locations.[a]

Chrysolina life stage	Roadside		Pasture		Blueberry field		Controls[b]	
	A	B	A	B	A	B	i) without *Chrysolina*	ii) with *Chrysolina*
	% infected plants							
First/Second instar	0	0	2	10	2	0	0	88
Third/Fourth instar	0	2	0	0	3	36	0	84
Adults	4	0	0	8	8	10	0	100

[a] Cell sizes were not adequate to perform Chi-square analysis. Values are derived from means of assessments on 50 plants 20 d after exposure to field-collected *Chrysolina.*

[b] Controls were (i) plants kept in cages without exposure to *Chrysolina* and (ii) plants exposed to *Chrysolina* that had fed for 2 d on heavily infected St. Johnswort plants under controlled conditions.

mo in contrast to 2 to 2.5 mo of active *Chrysolina* feeding. There was also no relationship between the presence or absence of *Chrysolina* and the subsequent survival of the weed (Table 4). Hence, we conclude that *Chrysolina* herbivory alone has little effect on St. Johnswort in Nova Scotia.

In contrast, shoot mortality was closely associated with the level of *Colletotrichum* infection (Table 3), and it closely mirrored the development of disease (Figure 2). Infection also reduced winter survival. No infected seedlings survived the winter, and depending on site, 4 to 49% of mature plants that were infected in October 1993 did not survive until spring (data not shown).

Models for *Colletotrichum* dispersal have focused on wind-driven rain splash (Yang and TeBeest 1992b). However, conidia produced in a gelatinous matrix are also suited to dispersal by physical contact with insects (Libby and Ellis 1954; Nemeye et al. 1990; Peña and Duncan 1989) or other agents (Yang and TeBeest 1992a). All stages of field-collected *Chrysolina* were contaminated with and could transmit *C.g.-hypericum* to healthy plants (Table 6). Numbers of contaminated *Chrysolina* may have been greater had collections been made only when conditions favored sporulation. *Chrysolina* were often observed feeding in or around stem lesions, and their movement would distribute conidia on contaminated body parts (Figure 3). Under controlled conditions, contaminated adults were effective in transmitting the pathogen to healthy plants (Tables 5–7). Conditions favoring inoculation and infection, as well as *Chrysolina* activity, would affect efficiency of *C.g.-hypericum* transmission. Adults may selectively transmit the pathogen to isolated weed stands and may account for the widespread occurrence of the disease in Nova Scotia.

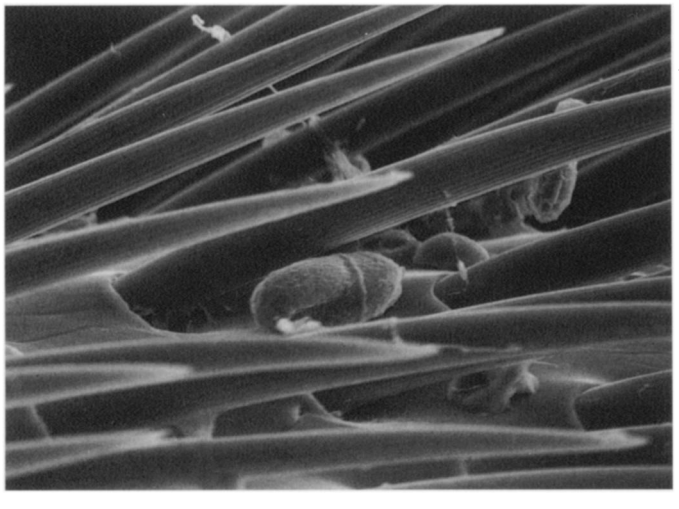

Figure 3. Scanning electron micrograph (×1500) showing a *Colletrotrichum* conidium lodged among the setae of a tarsus pad of a *Chrysolina hyperici* adult collected in the field from a *C.g.-hypericum*-infected plant.

We propose that *C.g.-hypericum* could potentially augment biological control of St. Johnswort elsewhere, especially in cooler, moister habitats. Inoculation through feeding wounds may help reduce the moisture requirements for infection even on dry sites (Auld and Morin 1995). The nonwoody, procumbent basal stems are susceptible to *C.g.-hypericum,* and infection of the crown may kill the weed directly or lessen its ability to survive unfavorable conditions. The co-use of pathogens with insects in biological control has not been exploited, but its potential has been cited (Charudattan 1986; Wilson 1969).

Releasing *Chrysolina* adults contaminated with conidia under favorable conditions was an effective method of introducing the disease (Table 7). Inducing acervuli formation on PDA under continuous light is a simple method of producing inoculum for insect contamination. Alternatively, it may be possible to simply homogenize the cultures in water and apply the inoculum directly to St. Johnswort hosting *Chrysolina* in the field.

In Nova Scotia, *C.g.-hypericum* appears to persist in uncultivated habitats as a saprophyte on diseased plant residues, in infected seeds, or as a pathogen on successive cohorts of infected St. Johnswort. We have noted that after 5 yr, St. Johnswort has not reestablished on former mycoherbicide plots. Like other *Colletotrichum*

Table 7. Status of St. Johnswort plants 6 wk after an out-of-doors 2-d exposure to *Chrysolina* adults contaminated with *C.g.-hypericum.*

Treatment	Status of plants						Mean ratings[a]
	1994			1995			
	Healthy	Infected	Dead	Healthy	Infected	Dead	
	%						
Contamination method[b]							
PDA culture	21	4	75	27	27	46	1.37 a
Infected plants	40	7	53				1.12 a
Control							
Sterile PDA	97	0	3	99	0	1	0.04 b
Healthy plants	3	2	5				0.11 b

[a] Means of categorical ratings of 0 (healthy), 1 (diseased), and 2 (dead). Means followed by the same letter are not significant by a *t* test at $P \leq 0.05$.

[b] Contamination method: adults were placed on either diseased plants (2 d) or sporulating *C.g.-hypericum* cultures on PDA (20–30 s) before being transferred to test plants. For control treatments, adults were placed on healthy plants or sterile PDA prior to transfer.

species (Waller 1992), *C.g.-hypericum* can latently infect a number of forage and weed species where it can sporulate on necrotic tissue under favorable conditions (Jensen and Doohan 1994). Lack of inoculum persistence has been cited as a constraint to natural epidemics of *Colletotrichum* in annual, arable crops, hence the need for inundative inoculation (Templeton et al. 1979). However, the ability of *C.g.-hypericum* and related species to persist suggests that *Colletotrichum* spp. may have potential as control agents when introduced into stable habitats.

C.g.-hypericum likely originates from the native *Hypericum* flora. Epidemics of *Colletotrichum* anthracnose often occur on both *H. canadense* and St. Johnswort cohabiting at the same site, and isolates are cross-virulent (Hildebrand and Jensen 1991). There was little difference in susceptibility to the pathogen of St. Johnswort selections from across North America (Jensen and Doohan 1994) and Australia (R.C.H. Shepherd, personal communication). Although natural enemies of weeds have generally been sought in their geographic origin, it appears that virulent pathogens of introduced weeds may also be found within the native flora. Other examples of introduced weeds that have shown susceptibility to native *Colletotrichum* spp. may include *Xanthium spinosum* in Australia (Butler 1951), *Malva pusilla* in western Canada (Mortensen 1988), and *Hakea sericea* in South Africa (Morris 1982). *Colletotrichum* spp. have received most attention as mycoherbicides with potential commercial application but may also find application in the context of classical or augmentative biological control. The co-use of host-specific insects in the selective transmission of the pathogen may help to minimize problems associated with its dissemination and infection by inoculum.

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