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Research Article

Assessing the Utility of a Native Pathogenic Fungus as a Biocontrol Alternative to Herbicide on Invasive Buckthorns in Forests of Upper Michigan

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ABSTRACT

Common buckthorn (*Rhamnus cathartica* L.) and glossy buckthorn (*Frangula alnus* Mill.) are considered invasive plants in North America, capable of displacing native vegetation. Their invasion often results in decreased biodiversity and weakened ecosystem resilience. Therefore, their removal is important for forest restoration. Invasive buckthorns are often managed using chemical herbicides and manual removal. We sought to determine the efficacy of *Chondrostereum purpureum* (a naturally occurring basidiomycete in North America) as a biocontrol agent for the treatment of invasive buckthorns in Upper Michigan, USA. We compared application treatments of *C. purpureum* and glyphosate to cut stump and girdled *R. cathartica* and *F. alnus* stems, to gain a better understanding of each treatment's relative impact on buckthorn regrowth in forested ecosystems of the Keweenaw Peninsula. We girdled or cut 300 buckthorn trees and applied either *C. purpureum*, glyphosate, or no treatment (control). One year after treatment applications, apparent tree mortality (i.e., zero stump sprouts) as proportion of total number of trees per treatment was highest for the glyphosate cut (94%) and girdle (81%) treatments and lowest for the controls (cut = 11% and girdle = 8%). Apparent mortality for *C. purpureum* treatments was 51% for cut surface and 67% for girdle applications. The *C. purpureum* treatments significantly reduced buckthorn stump sprouting compared with control treatments in just one growing season ($P < 0.001$) and showed statistically comparable stump sprout reduction with that of glyphosate treatments. Glyphosate applied to girdled stems showed similar success in reducing stump sprouts as *C. purpureum* applied to cut trees and to girdled trees ($P \geq 0.238$). Additionally, we found that percent canopy cover was significantly related to treatment success ($P = 0.013$), but that the influence of percent canopy varied among treatments. These findings suggest that *C. purpureum* application to buckthorn trees could provide land managers with an effective alternative or complement to traditional control techniques.

Index terms: chemical alternative; *Chondrostereum purpureum*; invasive plants; myco-biocontrol; silverleaf disease

INTRODUCTION

Glossy buckthorn (*Frangula alnus* Mill.) and common buckthorn (*Rhamnus cathartica* L.) are recognized as undesirable, invasive, woody plant species in North America (Gucker 2008; Zouhar 2011). Multiple factors lead to the dominance of invasive buckthorns in invaded ecosystems, including high fecundity and prolific growth rates, which enable them to form dense thickets. This, in combination with a generally longer growing season than some native plants, allows invasive buckthorns to displace native plant communities, including overstory tree regeneration (Boudreau and Wilson 1992; Knight et al. 2007). In addition to having negative effects on neighboring native plant species, buckthorn leaves and berries create nitrogen-rich soils where leaf litter falls, creating favorable conditions for earthworm invasion (Heneghan et al. 2007; Heimpel et al. 2010). In areas where invasive buckthorns and earthworms overlap, a positive feedback loop is created in which the presence of one helps to foster favorable conditions for the other (Heneghan et al. 2007; Knight et al. 2007). This co-facilitation further perpetuates their invasion while creating unsuitable conditions for native plant regeneration, and generally adversely impacting biodiversity and multiple trophic levels in forests (Klionsky et al. 2011; Roth et al. 2015; Schuh and Larsen 2015). The net effect of these buckthorn-driven ecosystem changes is decreased biodiversity and long-term, weakened ecosystem resilience.

Given the potentially deleterious and long-lasting effects of invasive buckthorns on forest ecosystems where present, their removal is often a critical component of forest restoration activities. The application of chemical herbicide is a common management practice because buckthorn prolifically resprout after cutting. However, chemical herbicide use on buckthorn often involves repeated applications. Additionally, using herbicide as a buckthorn management strategy is often costly, and could have environmental consequences (Delanoy and Archibold 2007; Annett et al. 2014; Florencia et al. 2017). Laborious repeated herbicide application is often required for removing well-established buckthorn colonies. Alternatively, manual removal of buckthorn, which involves removal of much of the root stock, can be a time- and labor-intensive process. Therefore, finding an alternative to chemical use and manual removal for buckthorn management is desired. A myco-biological control agent could meet these needs.

Chondrostereum purpureum (Fr.) Pouzar (Polyporales, Meruliaceae), a basidiomycete that is naturally occurring in temperate zones of North America, can act as a pathogen and saprotroph in sapwood of trees (Becker et al. 2005; Reina et al. 2019). It has broad-spectrum pathogenicity toward a variety of hardwood species (approximately 190 species on record), causing silverleaf disease primarily in plants in the Rosaceae family, although it is reported as low risk for nontarget infection associated with its application (Becker et al. 1999). *C. purpureum* usually enters its host through a fresh wound, cut stump, or stem lesion (Brooks and Moore 1925; Rayner and Boddy 1986; Spiers and Hopcroft 1988). The fungus grows through the xylem tissue of the tree, causing necrosis of the cambium, decay, and sometimes results in host death (Rayner 1977; Wall 1986; Wall 1991). Once entering the xylem, *C. purpureum* kills xylem parenchyma and uses the carbohydrates and nitrogenous compounds (Sinclair and Lyon 2005). The use of *C. purpureum* as a mycological, biological control agent has been tested on a variety of species, yet much is still unknown about its efficacy for invasive species management, including what ranges and climates it is most effective in, species effectiveness, and other practical applications of interest to potential managers.

Herein, we extend findings from previous *C. purpureum* studies to include both *R. cathartica* and *F. alnus* in northern Michigan mixed coniferous and deciduous forests. Our objective was to investigate the efficacy of *C. purpureum* as a biological control agent for treatment of both species of invasive buckthorns by comparing application treatments of *C. purpureum* and glyphosate to cut stump and girdled buckthorn stems. Based on earlier studies with other woody plants, we hypothesized that *C. purpureum* would colonize the stems of *R. cathartica* and *F. alnus* and inhibit regrowth through decay. Our specific hypotheses were that (1) control trees would be the most prolific stump sprouters, (2) *C. purpureum*-inoculated trees would have less than or equal to the number of basal resprouts compared to glyphosate-treated trees, and (3) girdled buckthorn trees inoculated with *C. purpureum* would have lower number of resprouts compared with cut stump buckthorn trees inoculated with *C. purpureum* after one growing season, based off findings from Au and Tuchscherer (2014).

METHODS

Study Sites

The study area includes 10 sites (five sites per buckthorn species) within Houghton County, Michigan, USA (Figure 1) (average annual precipitation: 84 cm; average spring/summer temperature: 8–20°C; Climate-Data.org). These sites are located on the Michigan Tech Trails and Recreational Forest, the Swedetown Trails, and the Central Houghton Greenspace—primarily public lands and recreational trails that are heavily used by the public and have been rapidly transformed in the last 20 y by buckthorn infestations. These study sites are located in northern mesic and boreal forests with a mix of ash (*Fraxinus* spp.), maple (*Acer* spp.), northern red oak (*Quercus rubra*), and northern white cedar (*Thuja occidentalis*) trees present. Each site contains six treatment plots (girdle with *C. purpureum*, girdle with glyphosate, girdle with no inoculum, cut with *C. purpureum*, cut with glyphosate, and cut with no inoculum), consisting of five trees per treatment (30 trees per site and 300 trees total). Before treatment applications, each treatment tree was labeled and measured for basal diameter, height, and whether it was multi-stemmed (with a common bole forked aboveground, but below 10 cm). Additionally, percent canopy cover readings were taken at approximately breast height for each treatment buckthorn tree following treatment application. Percent canopy was determined using CanopyApp (University of New Hampshire 2018; Durham, New Hampshire, USA), which provides comparable outputs to a densiometer (Laufenberg et al. 2020). Using a smartphone's gyroscope, canopy photos were taken level to the ground to ensure accurate readings. Leaf colors were selected by touch, and percent canopy was analyzed.

Inoculation Preparation

An isolate of *C. purpureum* was collected on 6 November 1994 from *Populus tremuloides* in Houghton, Michigan, and has been maintained as a live culture on 2% malt media (2M) and stored at 4°C in between annual transfers as part of the culture collection in the Forest Microbiology Lab, Wood Protection Group, Michigan Technological University. Fungal mycelia from this sample were cultured on 2M. Inoculum was prepared by plating out hyphae on 2M for 10 d of growth at room temperature. For field application, *C. purpureum* and 2M inoculum was slurried (hand-stirred) together, and diluted 1:10 with DI water. The slurry was then transferred to sterilized plastic squeeze bottles and was stored at 4°C until needed for field trials. To verify that *C. purpureum* was active in the slurry, following Koch's postulates, an inoculation test was conducted using an Olympus BH-2 6V 20W microscope to verify anatomical features of *C. purpureum* mycelium grown from each test batch before use in the field. Distinguishing anatomical features of *C. purpureum* include evidence of clamp connections, and hyaline hyphae of 2–3 micrometers across.

The six treatment combinations in this study were girdled trees with either *C. purpureum* or glyphosate applications, and cut trees with either *C. purpureum* or glyphosate applications. Five girdled and five cut stems were also included to act as controls at each site.

Treatment with *C. purpureum* began in early summer 2020 (23–25 and 29 June 2020) when temperature ranges reached 15–25 °C, while treatment with herbicide AquaNeat (glyphosate) concentrated at 50% (1:1 herbicide and water) began in late summer 2020 (4 August 2020), based on findings from Au and Tuchscherer (2014). All buckthorn trees were selected from existing populations, and treatment trees were selected in the following manner: trees spaced greater than 1 m apart, and trees that received the same treatment were grouped near each other, while also taking into consideration >1 m distance between them, to reduce treatment interactions. Trees were girdled by removing a 5 cm wide band of bark to expose the sapwood, at 20 cm above ground level. Cut-stump trees were cut with a handsaw 10 cm above ground level. *C. purpureum* inoculations and glyphosate treatments were applied immediately after wounding by coating either the cut surface of the stump or the exposed sapwood after girdling. In October 2020 and August 2021, buckthorn trees were

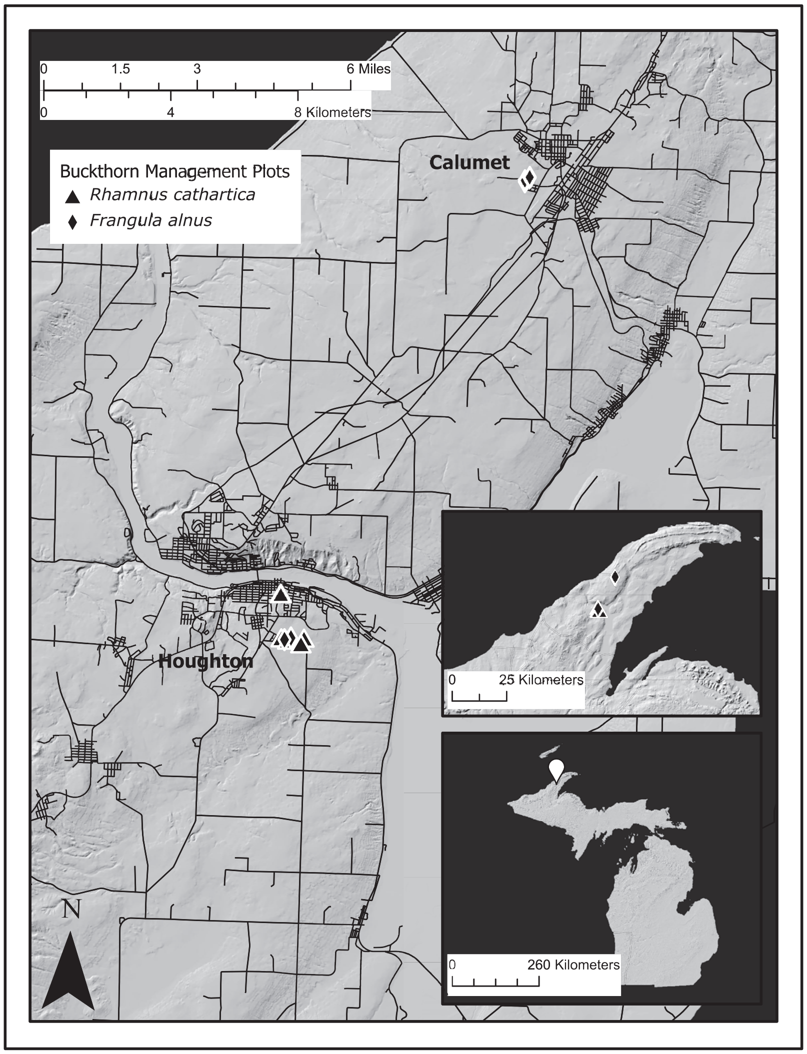

Figure 1.—Study sites located at Michigan Tech Trails and Recreational Forest, Houghton Greenspace, and Swedetown Trails in Houghton County, Michigan, USA; 2020 through 2021 field trials of fungal biocontrol and chemical herbicide treatment on invasive buckthorn trees. Triangle icons are sites infested with *R. cathartica* ($n = 5$) and diamond icons are *F. alnus* infested sites ($n = 5$).

evaluated for regrowth, and the number of stump sprouts (basal resprouts extending from the root collar and trunk) was recorded. Following Koch's postulates (Agrios 2005) to verify presence of *C. purpureum* in inoculated trees, a small hatchet was used to remove a chip of vascular xylem tissue from the base of 39 *C. purpureum*-treated buckthorn trees in August 2021. Tissue samples were plated on 2M to incubate for 10 d, and mycelial growth was examined under an Olympus BH-2 6V 20W microscope for the presence of *C. purpureum* anatomical features such as clamp connections and hyaline hyphae spanning 2–3 micrometers across (Sinclair and Lyon 2005). *C. purpureum* was successfully reisolated from 26 of the 39 tissue samples, verifying successful inoculation in the majority of trees.

Statistical Analysis

A generalized linear mixed model (GLMM) was employed using sprout count data from August 2021, with treatment as a fixed effect, and research site and buckthorn species as random effects. Basal diameter, tree height, percent canopy, whether a buckthorn tree was multi-stemmed (multistem), and treatment × percent canopy were examined as covariates. Pairwise comparisons were made using the Tukey method with an alpha = 0.05. Standardized residual plots were examined to confirm that model assumptions were met. A binary logistic regression was used to examine the likelihood of apparent mortality in 2021 (no sprouting) between treatments as a function of percent canopy. Pairwise odds ratios are presented for each treatment combination. Odds ratios greater than one indicate an increased likelihood and less than one a reduced likelihood of mortality. Ninety-five percent confidence intervals are provided, and intervals inclusive of values both less than and greater than one indicates a lack of evidence of a treatment effect. Regrowth comparisons were made using sprout count data recorded in October 2020 and August 2021 to assess how the amount of stump sprouting varies over time for a given treatment. Statistical analyses were performed using Minitab 20 software (State College, Pennsylvania, USA).

Table 1.—Full and reduced generalized linear models. Full model examines variables of basal diameter, multistem, tree height, percent canopy, buckthorn species, research site, treatment, and treatment × percent canopy. Variables that were statistically significant ($P < 0.05$) are in bold font.

Source	DF	*F*-value	*P*-value
Full Model: R-sq: 42.23%, R-sq(adj): 39.88%, R-sq(pred): 36.68%			
Basal Diameter	1	2.18	0.141
Multistem	1	2.24	0.136
Tree Height	1	0.56	0.453
Percent Canopy	**1**	**6.22**	**0.013**
Buckthorn Species	1	0.64	0.424
Site	1	0.41	0.521
Treatment	**5**	**35.91**	**<0.001**
Treatment × Percent Canopy	**5**	**6.43**	**<0.001**
Reduced Model: R-sq: 40.78%, R-sq(adj): 39.49%, R-sq(pred): 37.18%			
Percent Canopy	**1**	**9.03**	**0.003**
Treatment	**5**	**14.11**	**<0.001**
Treatment × Percent Canopy	**5**	**6.55**	**<0.001**

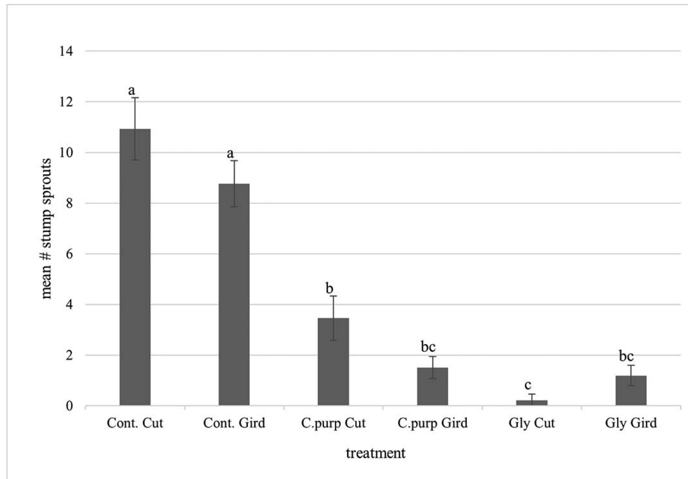

Figure 2.—August 2021 (1 year) mean number of stump sprouts present after one growing season for a given treatment (control cut, control girdle, *C. purpureum* [C.purp] cut, *C. purpureum* [C.purp] girdle, glyphosate [gly] cut, and glyphosate [gly] girdle). Standard error bars shown. Factors that are not significantly different at $P < 0.05$ from each other share the same lowercase letter(s) denoted above.

RESULTS

A total of 283 buckthorn trees were resurveyed from the 300 original trees included in this study. Eight *R. cathartica* and nine *F. alnus* trees were lost before final evaluations could be made, due to them either being mistakenly cut during other recreational or invasive species management activities in the area, or their flagging being removed. Mean stem diameter at 10 cm was 3.52 cm (min: 1.8 cm, max: 10.90 cm, $n = 283$). Mean tree height before treatments was 4.32 m (min: 1.44 m, max: 6.1 m, $n = 283$).

Treatment Comparisons

We compared six management treatments on two different buckthorn species across five sites per species, approximately one-year post-treatment. Treatment type was a highly significant fixed effect (Table 1, $P < 0.001$). Buckthorn species was not significant (Table 1, $P = 0.424$), thus, both species of buckthorn were combined for all remaining analyses. Wounding a tree by either cutting or girdling without additional application of either glyphosate or *C. purpureum* resulted in the most stump sprouts of all the treatments (Figure 2). *C. purpureum* application significantly reduced buckthorn stump sprouts compared with control treatments after just one growing season (Figure 2, $P < 0.001$). The *C. purpureum* treatment applied to girdled buckthorn trees and both glyphosate treatments were not significantly different from each other (Figure 2, $P \geq 0.823$). Glyphosate applied to girdled stems showed similar success in reducing stump sprouts as *C. purpureum* applied to cut trees and to girdled trees (Figure 2, $P \geq 0.238$). There was not a significant difference in stump sprouting between *C. purpureum* treatments applied to girdled and cut surfaces (Figure 2, $P = 0.426$).

One year after treatment applications, apparent tree mortality (i.e., zero stump sprouts) as proportion of total number of trees per treatment was highest for the glyphosate cut (94%) and girdle (81%) treatments and lowest for the controls (cut = 11% and girdle = 8%). Apparent mortality for *C. purpureum* treatments was 51% for cut surface and 67% for girdle applications. Binary logistic regression revealed a significant treatment effect ($P < 0.001$) and that the likelihood of apparent mortality increased with increasing canopy cover ($P = 0.043$). Treatment with *C. purpureum* increased the likelihood of apparent mortality for girdle and cut surface applications versus their respective controls (Table 2). The increase in the likelihood of apparent mortality, however, was greatest for herbicide treatments. Within *C. purpureum* and herbicide treatments, the likelihood of apparent mortality did not vary significantly between cut and girdle treatments (Table 2).

Table 2.—Logistic regression pairwise odds ratios assessing the influence of treatment combination on the likelihood of apparent mortality (no live sprouts in 2021). Odds ratios greater than one indicate an increased likelihood of apparent mortality and less than one a reduced likelihood. Ninety-five percent confidence intervals (95% CI) inclusive of values both less than and greater than one indicate that there was no evidence of a treatment effect.

Treatment Comparison	Odds ratio (95% CI)
C. purpureum cut vs. Control cut	7.97 (2.67, 23.82)
C. purpureum girdle vs. Control cut	16.22 (5.25, 50.14)
Control girdle vs. Control cut	0.65 (0.16, 2.62)
Glyphosate cut vs. Control cut	126.16 (28.05, 567.40)
Glyphosate girdle vs. Control cut	33.31 (10.15, 109.28)
C. purpureum girdle vs. *C. purpureum* cut	2.04 (0.87, 4.76)
Control girdle vs. *C. purpureum* cut	0.08 (0.02, 0.26)
Glyphosate cut vs. *C. purpureum* cut	15.83 (4.29, 58.44)
Glyphosate girdle vs. *C. purpureum* cut	4.18 (1.65, 10.56)
Control girdle vs. *C. purpureum* girdle	0.04 (0.01, 0.14)
Glyphosate cut vs. *C. purpureum* girdle	7.78 (2.06, 29.36)
Glyphosate girdle vs. *C. purpureum* girdle	2.05 (0.78, 5.38)
Glyphosate cut vs. Control girdle	193.82 (40.21, 934.20)
Glyphosate girdle vs. Control girdle	51.18 (14.38, 182.10)
Glyphosate girdle vs. Glyphosate cut	0.26 (0.07, 1.05)

Table 3.—GLM exploring treatment × percent canopy interaction to examine significance of percent canopy on treatment success for various treatment combinations on invasive buckthorns.

GLM: Treatment × Percent Canopy			
R-sq: 47.16%, R-sq(adj): 45.02%, R-sq(pred): 41.37%			
Source	Coef	SE Coef	*P*-value
***C. purpureum* cut**	**−0.1026**	**0.0331**	**0.002**
C. purpureum girdle	0.0508	0.041	0.216
Control cut	**−0.1495**	**0.0365**	**<0.001**
Control girdle	**0.0684**	**0.0336**	**0.043**
Glyphosate cut	0.0437	0.0421	0.29
Glyphosate girdle	**0.0894**	**0.0385**	**0.021**

In cambium samples collected to verify Koch's postulates, verified samples showed fewer mean stump sprouts compared with samples where Koch's postulates were unverified for both *C. purpureum* cut stump and girdle treatment combination. The mean number of stump sprouts was six in samples where Koch's postulates were unverified. In samples where Koch's postulates were verified, the mean number of stump sprouts was two.

Percent canopy was a significant covariant (coeff: −0.0585, $P = 0.013$). Additionally, the interaction between treatment and percent canopy was significant ($P < 0.001$). The treatment combinations significantly influenced by percent canopy were *C. purpureum* cut, control cut, and weakly significant herbicide girdle and control girdle (Table 3). As percent of canopy increased (denser canopies, lower light availability), treatment efficacy increased in *C. purpureum* cut and control cut buckthorn trees (Figures 3A and 3E). Contrastingly, in herbicide girdle and control girdle buckthorn trees, treatment efficacy decreased as percent canopy increased (Figures 3B and 3D). The interactions between percent canopy and *C. purpureum* girdle and herbicide cut treatments were not significant (Table 3, Figures 3C and 3F).

Stump sprouts were also assessed in October 2020 and compared to sprout counts from August 2021. The number of stump sprouts decreased by 46% for *C. purpureum* cut stump and 59% for *C. purpureum* girdling treatments between October 2020 and August 2021, showing a significant reduction in stump sprout counts for *C. purpureum* application to both cut and girdled trees between October 2020 and August 2021 ($P = 0.037$ and 0.001, respectively; Table 4). Sprout suppression did not increase over time for glyphosate application to cut or girdled trees, with the number of stump sprouts increasing by 100% in cut and 550% in girdling treatments between October 2020 and August 2021 (Table 4). Glyphosate application to girdled buckthorn trees showed a significantly higher amount of stump sprouting over the time period (Table 4, $P = 0.0025$), indicating less effectiveness over time.

DISCUSSION

In a study conducted by Wall (1990), fewer stump sprouts were reported in nine hardwood species treated with *C. purpureum* than in their uninoculated counterparts (control trees). Our study is the first known study that has investigated the efficacy of *C. purpureum* on both *R. cathartica* and *F. alnus* as a myco-biological control agent. We hypothesized that untreated control trees would be the most prolific stump sprouters, which was the case here. Overall, all *C. purpureum* and herbicide treatment combinations showed fewer stump sprouts than control trees and higher probabilities of apparent mortality. *C. purpureum* treatment when applied to girdled trees performed as well as both herbicide treatments in terms of number of sprouts, but less well in terms of apparent mortality as of 2021. Additionally, the *C. purpureum* cut treatment combination performed as well as the herbicide girdle treatment in terms of number of sprouts, but the herbicide girdle treatment was

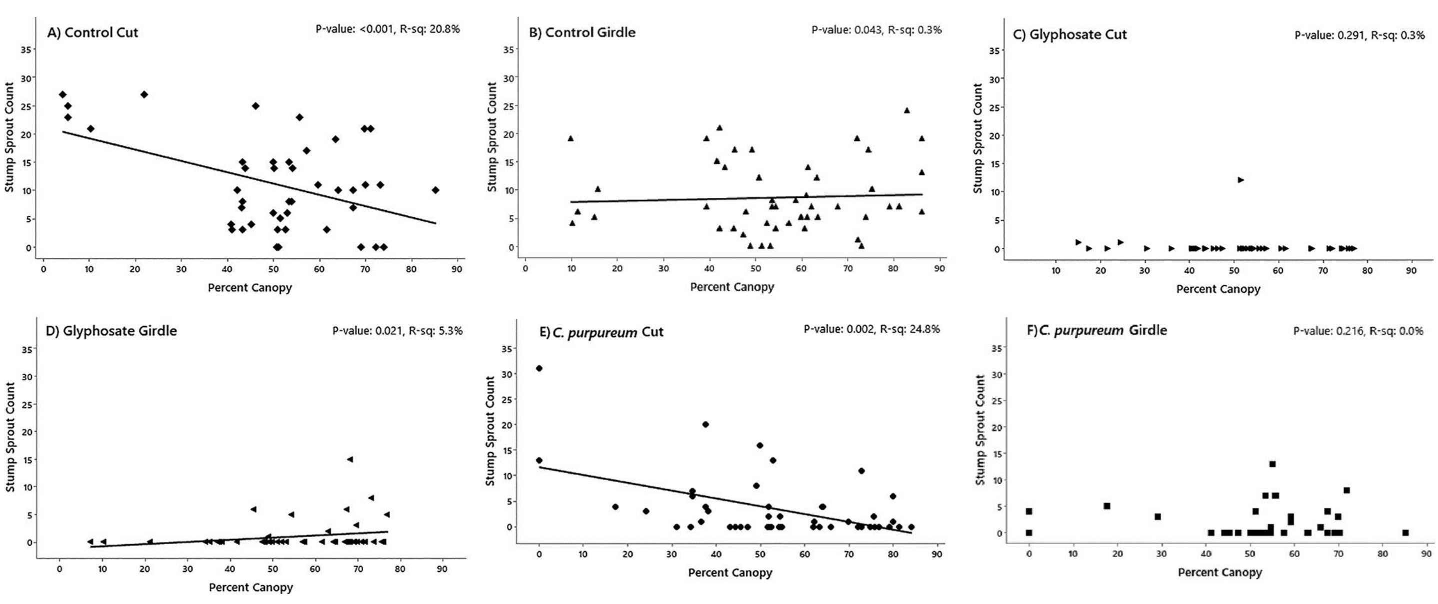

Figure 3.—A–F: Buckthorn stump sprout count versus forest percent canopy for treatment combinations, trend lines shown for treatment combinations in which percent canopy had a significant effect on treatment success.

Table 4.—Comparisons of buckthorn stump sprout data from October 2020 and August 2021 for a given treatment combination. Treatment combinations include cut or girdled buckthorn with *Chondrostereum purpureum* or glyphosate applications. Percent change was calculated by (Aug 2021 count – Oct 2020 count)/|Oct 2020 count|. Statistical significance between treatments ($P < 0.05$) is shown in bold font.

Treatment Combination	Oct 2020 avg. sprout count	Aug 2021 avg. sprout count	% change in stump sprouting over time (*P*-value)	Aug 2021 proportion of dead trees (%)
***C. purpureum* cut**	**6.4**	**3.5**	**−46 (0.037)**	51
***C. purpureum* girdle**	**3.7**	**1.5**	**−59 (0.001)**	67
Control cut	9.2	10.9	19 (0.298)	11
Control girdle	8.6	8.8	2 (0.934)	8
Glyphosate cut	0.1	0.2	100 (0.525)	94
Glyphosate girdle	**0.2**	**1.3**	**550 (0.025)**	81

four times more likely to result in apparent mortality as of 2021. In buckthorn trees treated with *C. purpureum*, Au and Tuchscherer (2014) saw fewer stump sprouts in girdled *R. cathartica* than in cut stump *R. cathartica* trees. We did not find a difference between girdling and cut stem application of either *C. purpureum*.

In our study, buckthorn trees that were inoculated with *C. purpureum* did not show a significant difference in treatment success whether they were cut or girdled. Consequently, our hypothesis, that girdled buckthorn trees inoculated with *C. purpureum* will have fewer resprouts compared with cut stump buckthorn trees inoculated with *C. purpureum*, is not supported. Overall, our results showed that both *C. purpureum* treatment combinations were not significantly different from girdle herbicide treatment in their efficacy in reducing stump sprouting in invasive buckthorns after just one season of monitoring, which would give land managers a degree of flexibility when using *C. purpureum* for buckthorn management.

Fewer average number of stump sprouts were found in samples where the fungus was verified in the stumps following Koch's postulates, suggesting that *C. purpureum* did not successfully establish in all treated trees. These trees stump sprouted in similar numbers to that of control treatments, further supporting the need to ensure successful inoculation conditions are met. We purposefully did not inoculate trees when rain was forecast within the following two days, however we noted a large presence of slugs and invertebrates on the slurry paste in the field on some trees soon after *C. purpureum* was applied: it is possible they consumed too much of the media and the fungus dried out before successfully penetrating the wood.

It has been shown that for many hardwood species a decrease in percent canopy results in an increase in stump sprouting (e.g., Gardner and Helmig 1997; Dey et al. 2007; Atwood et al. 2009). In smaller-diameter hardwoods (10–20 cm dbh), stump sprout growth can be prolific in high light environments, especially in clearcut areas (Dey et al. 2007). Increasing amounts of residual overstory trees suppress growth, displaying a negative relationship between overstory shade and hardwood sprout growth in mechanically wounded hardwoods (Dey et al. 2007). Gardner and Helmig (1997) examined the effects of thinning on stump sprouts of water oak (*Quercus nigra* L.), and showed that there was greater stump sprout survival after heavy thinning (60% reduction in basal area) compared to light thinning (40% reduction in basal area). Partial harvesting systems have also been shown to result in lower rates of sprouting than clearcutting in nine hardwood species (Atwood et al. 2009). Almost complete mortality of invasive buckthorns has been reported in canopies that permit ≤3% transmission of incoming light, with surviving buckthorn strongly tied to light availability (Schuster et al. 2020). This indicates a tight linkage between buckthorn performance and light availability, where decreased light availability reduces survival in invasive buckthorns (Schuster et al. 2020).

To our knowledge, canopy cover has not been examined in previous studies that examined the impacts of *C. purpureum* as a control on invasive buckthorns. Percent canopy was a significant variable in our statistical analyses; however, the influence of percent canopy varies by treatment combination. Treatment success in *C. purpureum* cut buckthorn was significantly related to percent canopy (Table 3). As percent canopy decreased (increasing available light on the forest floor), the efficacy of *C. purpureum* in cut stump trees also decreased. A higher percent canopy may be more beneficial for the *C. purpureum* cut stump treatment, as it provides a more humid, cooler microenvironment for fungal establishment and growth. It may be that the exposure of a cut stump to more direct sunlight and higher temperatures creates unfavorable conditions for *C. purpureum* growth in areas where daytime temperatures surpass *C. purpureum* limits (Au and Tuchscherer 2014). Temperatures exceeding 30°C may be too hot for *C. purpureum* establishment, as the mean maximum temperature for successful field application of *C. purpureum* is 27°C and the optimum temperature range for spore germination and fungal growth is 24–26°C (Sinclair and Lyon 2005). Additionally, greater light availability to the root crown and axillary buds of the cut stump may stimulate sprouting. This is the likely explanation for the negative relationship between percent canopy and stump sprouting in the control cut surface treatment. This interpretation is supported by the lack of relationship between percent canopy and sprouting of girdled trees inoculated with *C. purpureum* because their immediate canopies remain intact.

In contrast to the negative relationship between percent canopy and stump sprouting described above, there was a positive relationship between percent canopy and stump sprouting for the glyphosate with girdling treatments. In trees with higher percent canopy above, presumably from the trees being treated, net photosynthesis and carbon capture may be higher. A more productive buckthorn tree may overcome wounding better because it has more carbohydrates sequestered; therefore, girdling a more productive buckthorn may have a higher capacity of producing more stump sprouts compared with that of a less productive tree. However, more research would be beneficial to determine if this logic can be fully supported.

The mortality of other hardwood trees inoculated with *C. purpureum* has been reported as increasing from 92% to 100% a year following inoculation (Becker et al. 2005). Similarly, treatment efficacy of *C. purpureum* improved from October 2020 to August 2021 in our study. It is likely that this decrease in the number of buckthorn stump sprouts coincides with *C. purpureum* continuing to colonize and necrotize the buckthorn sapwood, further exploiting its resources and hindering vascular transport abilities as more time passed (Rayner 1977; Wall 1986, 1991). This suggests that future sprout count measurements will continue to decrease until host death occurs. Following these treatments for another 2–3 y will be important to determining lasting effects of either treatment combination with *C. purpureum*.

C. purpureum as a biocontrol mechanism for this purpose was not commercially available to the public in the United States or Canada at the time of this writing. We cultured our own isolate and prepared *C. purpureum* inoculum to carry out field trials. Without commercialization of this fungus, its application as a myco-biological agent is limited, as it would be inaccessible to most land managers. A patent for the use of *C. purpureum* as a biological control agent for weeds and invasive species is in effect (United States Patent 5587158), which further limits the accessibility and development of potential new commercial sources of *C. purpureum* at this time. While multiple companies in North America and Europe have offered it for sale in the past, it appears that none is currently on the market or being regularly produced for this purpose. Trends in pest management suggest a public preference for fewer chemical control options when possible (Fravel 2005), thus there is a likely potential market for this product.

Wounding a tree by either cutting it to a stump or girdling the stem, and subsequently applying fungal inoculum, was a time-consuming process, one that might not save land managers much time in comparison to using a chemical herbicide. However, the use of *C. purpureum* can alleviate concerns of chemical herbicide off-target species and water quality impacts, which may be especially important at sensitive sites. It may also be that *C. purpureum* can act as a longer-lasting control mechanism than the chemical herbicides, as it continues to live in the wood and grow through any belowground root tissue over multiple seasons. Testing different methods for wounding buckthorn trees and applying fungal inoculum, such as brush cutting and broadcast spraying on smaller stems, could be beneficial in potentially developing more time-efficient protocols for using *C. purpureum*. Future testing could expand to examine the efficacy of *C. purpureum* on other woody invasive species in similar temperate zones, such as invasive honeysuckles (*Lonicera* spp.), autumn olive (*Elaeagnus umbellata*), or Japanese barberry (*Berberis thunbergii*), which often co-invade with buckthorn.

CONCLUSION

C. purpureum inoculation treatments via girdling showed similar reduction of stump sprouting as that of glyphosate treatments via girdling or cut stump, in both *F. alnus* and *R. cathartica* species. Additionally, *C. purpureum* inoculation via cut stump showed similar reduction of stump sprout as that of *C. purpureum* and glyphosate treatments via girdling. Reduction of stump sprouts in *C. purpureum*-inoculated buckthorn was shown regardless of the species of buckthorn, the height and diameter of the tree, and whether it was multi-stemmed. Our results indicate that *C. purpureum* could provide a chemical-free alternative for buckthorn management when applied during the appropriate air temperature range. This potential myco-biological control alternative would act as a step forward to creating a more resilient, biodiverse ecosystem in places where invasive buckthorn species are currently present. Ideally, these findings could provide landowners and land managers with additional options for managing invasive buckthorns.

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