

Article

A Foliar Endophyte of White Spruce Reduces Survival of the Eastern Spruce Budworm and Tree Defoliation

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Abstract: Wild eastern spruce budworm (*Choristoneura fumiferana* Clemens) were reared on white spruce (*Picea glauca* (Moench) Voss) trees, half of which had been previously inoculated with a native endophytic fungus, *Phialocephala scopiformis* DAOM 229536 Kowalski and Kehr (Helotiales, Ascomycota). Survival up to pupation and up to adult emergence was approximately 27% higher for budworm juveniles that developed on control trees compared to trees inoculated with the endophyte. The endophyte did not influence the size or sex of survivors but did reduce defoliation by approximately 30%. Reductions in defoliation on endophyte-inoculated versus control trees, due to reductions in survival of juvenile budworms, suggests that tree inoculations with *P. scopiformis* could play an important role in integrated management programs against the eastern spruce budworm.

Keywords: endophyte; *Phialocephala scopiformis*; *Picea glauca*; spruce budworm; defoliation

1. Introduction

Mutualisms between fungi and plants have been reported for most plant groups [1–3]. Associations between endophytic fungi that live inside the needles (hereafter endophytes) of conifers are common [4–6]. These infections are known to contribute to increased tree tolerance to herbivores, such as insect pests [3,7]. Although the role of endophytic fungi in increasing plant tolerance to insects is well studied in cool season grasses (Rodriguez et al., 2009), decreases in insect pest performance attributable to an endophytic fungus has only been documented for one defoliating insect on a conifer.

Several studies carried out under laboratory conditions or in a nursery reported that endophytes reduced the size or survival of the eastern spruce budworm, *Choristoneura fumiferana* Clemens (hereafter budworm) [8–12]. In a manipulated field experiment carried out with larvae from a laboratory colony, we showed that the rugulosin-producing endophyte, *P. scopiformis*, DAOMC 229 in the Canadian Collection of Fungal Cultures, Agriculture AgriFood Canada, Ottawa, Canada, 536 Kowalski and Kehr (Helotiales:Ascomycota), a native endophytic fungus isolated from white spruce in eastern Canada [9], reduced the survival of budworm developing on white spruce (*P. glauca* (Moench) Voss) trees that had been inoculated with the endophyte more than 10 years earlier [13]. Reductions in budworm survival were highest for larvae developing in the mid and upper crown of trees, the most important crown region for photosynthesis and tree growth. In a subsequent field study, the survival of both wild budworm and budworm from a laboratory colony was reduced when forced to develop on trees inoculated with *P. scopiformis* compared to non-inoculated control trees [14].

The budworm is the most important forest pest in eastern Canada (MacLean 2016). Outbreaks often last 10 years [15,16], causing high levels of tree mortality and large reductions in growth of surviving trees [17]. Results from the manipulative studies described above suggest that inoculation of white spruce trees with *P. scopiformis* could improve tree performance in a budworm outbreak if reduced budworm survival resulted in lower levels of host tree defoliation. Both the growth and mortality of budworm host trees, spruce and fir, are directly related to the amount of defoliation caused by budworm larvae [18,19].

Here, we present results from a manipulated field experiment carried out with wild budworm that were forced to develop on white spruce trees that were either previously inoculated with the native endophyte, *P. scopiformis*, or on control (i.e., uninoculated) trees. We tested the prediction that budworm survival and associated defoliation would be lower on endophyte-inoculated trees.

2. Materials and Methods

2.1. Study Site, Tree Selection, and Experimental Design

To obtain wild, overwintered second-instar larvae for use in field studies in the spring of 2016, we collected shoots bearing spruce budworm eggs from spruce trees in southeastern Quebec, near Causapsal, in July, 2015. Egg-bearing shoots were cut from branches and transported in coolers to our laboratory in Douglas, New Brunswick. The shoots were placed in metal trays and reared at 22 ± 1 °C and $65 \pm 5\%$ RH under a 14 L:10 D photoperiod. A piece of Parafilm™ with a smaller piece of cheesecloth attached to it had been placed on the bottom of each tray and another larger piece was used to seal the top of each container. A black piece of cardboard with a 12 cm × 6 cm rectangular hole in the center was placed over each tray. Following egg hatching, the first instar budworm larvae spun hibernacula on the cheesecloth. The pieces of cheesecloth were removed two weeks later and placed in sleeve cages. We affixed the sleeve cages to the lower boles of spruce trees located adjacent to the laboratory and removed them when needed for experiments in spring 2016.

The study was carried out on the privately owned J.D. Irving, Limited, Black Brook district in northwestern New Brunswick (latitude 47°15'51" N, longitude 67°41'14.6" W) in two adjacent "test plots" of 7-year-old white spruce, *Picea glauca*, planted from seedling stock in 2009. Trees were planted at 2.1 m × 2.1 m spacing, with a total of approximately 3000 trees in each plot. The plantation area was very uniform in terms of aspect, soil characteristics, terrain uniformity, and soil depth. Half of the trees were inoculated twice in the nursery (separated by 2 weeks) with insect-toxin-producing *P. scopiformis* cultures in 2008 when the trees were approximately 6 cm in height. The other half of the trees were not inoculated.

In autumn 2015, we selected control and endophyte-inoculated trees along north-east to south-west transects throughout the adjacent test plots within a total radius of 100 m and identified them with flagging tape. To verify that endophyte-inoculated and control trees did and did not, respectively, contain *Phialocephala scopiformis*, 15 current-year shoots were collected from the mid-crown of each tree, placed in a bag (one per tree), and transported in a cooler to a −16 °C freezer. The samples were held in the freezer until they were processed. Branches were freeze-dried and the needles separated from stem material. Needles were ground and sent to Carleton University, where the insect toxin rugulosin was measured by HPLC [20].

In early May 2016, we selected 32 pairs of study trees, where one tree in each pair had tested positive for the presence of the endophyte and one had tested negative. Trees with noticeable mechanical damage, browsing, defoliation, or deformation due to spruce gall midge or spruce bud midge were excluded from the study. Before placement on study trees, wild budworm that had overwintered in Douglas, N.B., were removed from sleeve cages and stored at 4 °C for 1–2 weeks. Pieces of cheese cloth, on which the second instars had previously spun hibernacula, were placed at 20 ± 1 °C, 75% RH under a 14 L:10 D photoperiod until the first larva emerged. Pieces of cheesecloth with 5, 8, or 12 hibernacula were then cut under a binocular microscope and transported to the field in a cooler.

To evaluate the influence of the endophyte and larval density on budworm performance, we selected four easterly-facing mid-crown branches on each tree and marked each branch with flagging tape. Each branch was randomly assigned to one of two phenology and larval density levels. One piece of cheese cloth containing second-instar spruce budworm larvae inside hibernacula was placed on the underside of each branch, attached with a small pin, and covered with a sleeve cage, as in [13]. Five or 12 larvae were placed on branches on 18 June when buds were swelling but had not burst, and five or eight larvae were placed on branches on 21 June when almost all buds had burst, due to unseasonably hot temperatures during the intervening three days. We had originally planned to place five or 12 larvae on both dates but had to reduce the number of larvae on higher density branches to eight during the second phenology date due to a lack of vigorous insects. This precluded us from testing the interacting effects between the endophyte and phenology or larval density but did allow us to determine the overall impact of the endophyte under a range of conditions (see statistical analysis below).

The piece of cheese cloth was removed from each sleeve cage two weeks later and the number of larvae that did not emerge from hibernacula was determined under a binocular microscope. Only the numbers of larvae that had emerged from hibernacula were included in calculations of survival. Sleeve cages were monitored weekly until the first pupa was observed. Thereafter, sleeve cages were examined every 3–5 days and any pupae were removed, placed on moistened vermiculite in aerated containers, and returned to the laboratory where they were reared at 20 ± 1 °C, $75 \pm 5\%$ RH under natural light. Containers in the laboratory were examined daily and the vermiculite was moistened as needed. All emerged adults were sexed, killed by freezing, and then one forewing of each female was removed and placed under a cover slip on a microscope slide. The length of each forewing was subsequently measured, using a micrometer in a binocular microscope. Sleeve cages were removed after all larvae had died or pupated. Defoliation on current-year branches was visually estimated at the end of summer using 10% categories, as in [21].

2.2. Statistical Analysis

Data were pooled at the tree level to determine the overall effect of the endophyte-given variability in larval emergence or budburst phenological synchrony and in larval density. The influence of the endophyte on the larval survival (i.e., second instar to pupation), total survival (i.e., second instar to adult emergence), and adult sex ratio was evaluated using one-way generalized linear models with logit link functions and binomial probability distributions. A linear model was used to examine the effects of endophyte on the wing length of female survivors and on defoliation. A linear correlation model was used to examine the relationships between defoliation and larval and total survival. Analyses were carried out using the *glm*, *lm*, and *cor.test* functions of R [22]. We subjected defoliation estimates, which were non-count proportion data, to logit transformation before analysis; we used the “empirical logit”, $\log[(y + \epsilon)/(1 - y + \epsilon)]$, where ϵ is the smallest non-zero proportion observed, because our data included values of 0 and 1 [23].

3. Results and Discussion

Survival of spruce budworm larvae up to pupation (larval survival) and adult emergence (total survival) was significantly lower on endophyte-inoculated than control trees ($z \geq 3.644$, $p < 0.001$). Both larval and total survival were approximately 27% higher for budworm developing on control than on endophyte-inoculated trees (Figure 1). There were no differences in the sex ratios ($z = 1.567$, $p = 0.117$) or female wing lengths of survivors ($F = 0.485$, $p = 0.391$) that developed on endophyte-inoculated or control trees.

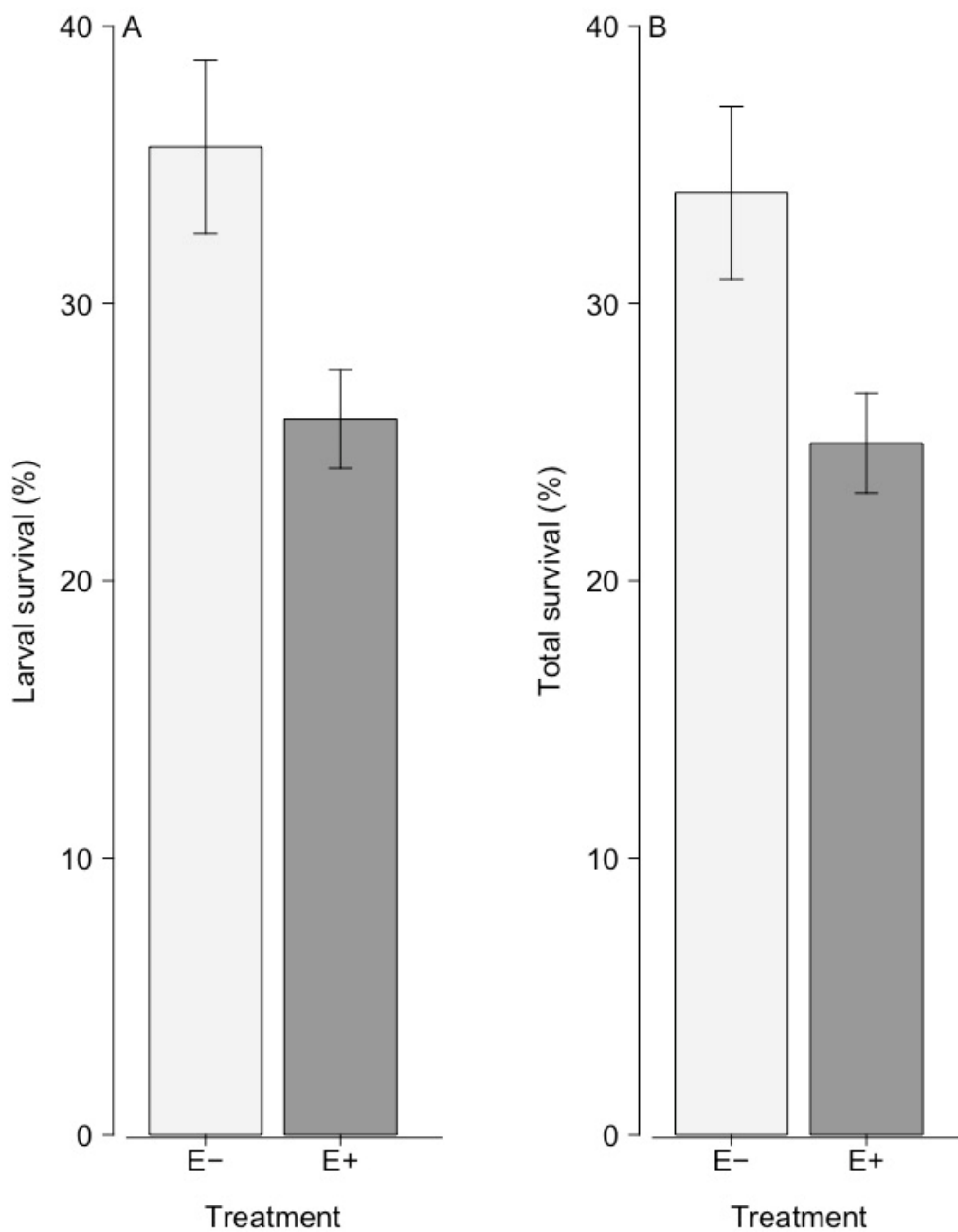


Figure 1. Mean (\pm SE) larval (i.e., second instar to pupa) (A) and total (i.e., second instar to adult emergence) (B) survival of eastern spruce budworms reared on white spruce trees. Thirty-two trees contained a native endophytic fungus (E+, dark bars) and 32 control trees did not (E-, light bars).

These results are similar to those observed in two previous field studies [13,14], where *P. scopiformis* significantly reduced budworm survival but did not influence the wing lengths or sex ratio of survivors. As in those studies, most of the higher mortality incurred by budworms developing on endophyte-inoculated trees occurred during larval development.

Reductions in budworm survival in the present study occurred over a range of budworm densities and variable spring emergence or budburst phenology conditions. The negative impact of the endophyte on budworm development can be influenced by interactions with many factors, such as insect density and weather [13]. Although we were unable to statistically examine any such interactions, if they did occur, the significant impact of the endophyte on budworm development

and defoliation over a range of conditions in the present study supports results from earlier field studies [13,14].

Defoliation was significantly lower on endophyte-inoculated than control trees ($t = 2.875$, $p = 0.0055$). Mean defoliation levels were low but were approximately 30% higher on control than on endophyte-inoculated trees (Figure 2). Defoliation was positively related to both larval ($r = 0.57$, $t = 5.523$, $p < 0.0001$) and total ($r = 0.58$, $t = 5.572$, $p < 0.0001$) survival, and thus reductions in defoliation on endophyte-inoculated trees were associated with reductions in budworm survival. To the best of our knowledge, this is the first field study to demonstrate that an endophytic fungus can decrease defoliation in a conifer.

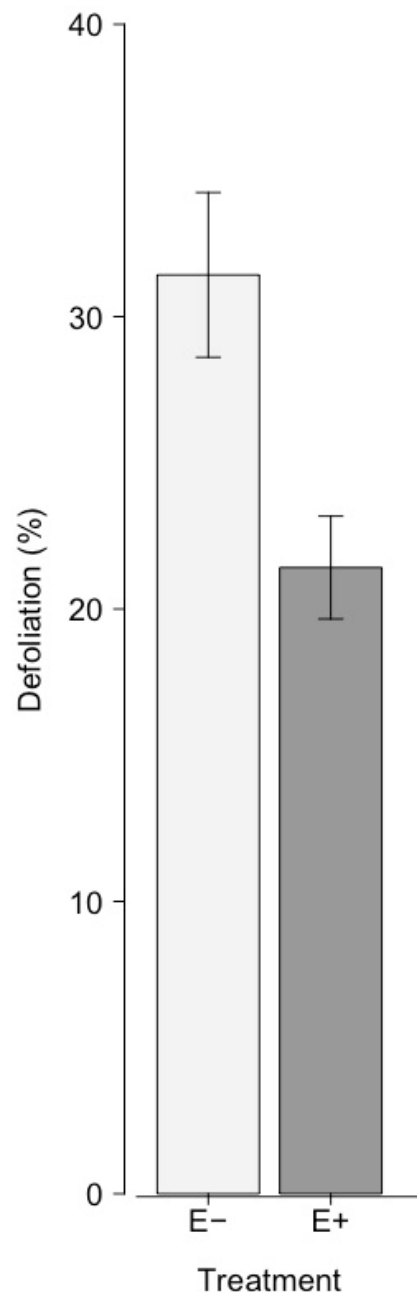


Figure 2. Mean (\pm SE) defoliation of white spruce trees by eastern spruce budworm. Thirty-two trees contained a native endophytic fungus (E+, dark bars) and 32 control trees did not (E-, light bars).

Mean defoliation levels were relatively low (<30%) in the present study, and thus it is noteworthy that reduced levels of defoliation on endophyte-inoculated versus control trees were statistically

significant. We speculate that the significant reduction in defoliation observed in this, but not the two previous studies [13,14], where percent defoliation averaged 18–25%, was due in part to budworm larvae dying earlier in development on endophyte-inoculated trees. Temperatures during the three days between the placement on experimental trees of the first and second cohorts of second-instar larvae were unseasonably hot, with high temperatures of 27.0 °C, 30.5 °C, and 30.2 °C on June 18, 19, and 20 at the nearest weather station, 17 km from the study plots [24]. The greatest difference in budworm mortality on endophyte-inoculated versus control trees occurred in the first cohort, the only cohort subjected to the high temperatures. Thus, it is possible that many budworm larvae in the first cohort placed on endophyte-inoculated trees died shortly after colonizing experimental trees.

Reactive management, primarily by aerial insecticide application during outbreaks, was the primary tactic used in previous budworm outbreaks [25]. In contrast, there has recently been an increased emphasis on early intervention, via application of biological insecticides, to prevent low-density but growing budworm populations from attaining outbreak densities [26]. This approach has resulted in early success in a large area-wide management research project, where spruce and fir stands experienced lower levels of defoliation [25]. Inoculation of white spruce trees with *P. scopiformis* is a proactive tactic that may further reduce defoliation under a variety of environmental conditions.

4. Conclusions

This is the first field study to show that a naturally occurring endophytic fungus can reduce defoliation in a conifer. In previous field experiments, we reported that the survival of the most important defoliating pest in North American temperate forests, the spruce budworm, was reduced when feeding on host trees containing a native endophytic fungus, *P. scopiformis* [13,14]. In the present study, reductions in budworm survival on trees containing the native endophyte versus control trees without the endophyte also resulted in reduced defoliation. These results demonstrate that endophytic fungi can protect conifers from insect defoliators.

Author Contributions: D.Q. and J.D.M. contributed to the general study conceptualization. J.D.M. and D.Q. supervised and conducted the laboratory or field observations and measurements. D.Q. and S.E. carried out the statistical analyses and made figures. D.Q. was responsible for funding acquisition and project administration. D.Q., G.A., A.M., S.E., J.D.M. interpreted the data and contributed substantially to manuscript preparation, development, and revision. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: G.A. and A.M., respectively, are employees of J.D. Irving, Limited (JDI), and Maritime Innovation, Limited (a wholly owned subsidiary of JDI). The company provided funding for this research.

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