An association between invasive apple clearwing moth, *Synanthedon myopaeformis* (Lepidoptera: Sesiidae), and necrotic cankers on *Malus domestica* (Rosaceae) trees in British Columbia

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ABSTRACT

Sudden apple decline is a complex disease that causes rapid mortality in apple trees across North America and is typically associated with a necrotic canker near the graft union. To date, causal factors have been diverse and speculative, and infestation with *Synanthedon myopaeformis* (apple clearwing moth) was identified by producers in British Columbia as a concern related to the disease. Assessments of declining orchards in 2019 showed an association between incidence of necrotic cankers and infestation of *S. myopaeformis*. Observations of tree stems 15 cm above the graft union showed that 100% of signs of *S. myopaeformis* infestation (pupal casings, exit holes, frass) were in necrotic tissue. A positive correlation was identified between necrotic canker size and number of *S. myopaeformis* signs. It is unlikely that *S. myopaeformis* infestation is the underlying cause of sudden apple decline, but this observational field investigation suggests a relationship between infestations and presence of necrotic cankers, which may be an additional stressor contributing to apple tree collapse associated with sudden apple decline.

Keywords: apple clearwing moth, red-belted clearwing moth, sudden apple decline, rapid apple decline

INTRODUCTION

Synanthedon myopaeformis Borkhausen (Lepidoptera: Sesiidae) (apple clearwing moth) was first detected in British Columbia, Canada in 2005 in the Similkameen Valley (Philip 2006); however, a recent review and DNA barcoding of local arthropod collections by Nelson and Moffat identified a specimen collected in 2003. Native to Europe, where it is known commonly as red-belted clearwing moth, the larvae bore into vulnerable tissue such as within burr knots or cracked bark, often around the graft union, before pupating and emerging as adults. Improved monitoring strategies (Aurelian *et al.* 2012, 2015; Eby *et al.* 2013; Judd and Eby 2014) have led to increased detections in British Columbia's Similkameen and Okanagan Valleys. Despite various investigations into management strategies, including biological and chemical controls (Cossentine *et al.* 2010; Judd *et al.* 2015), the regional apple industry has yet to effectively manage this invasive insect.

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Recently, infestations of *S. myopaeformis* and *Scolytus rugulosus* Müller (Coleoptera: Curculionidae: Scolytinae) (shothole borer beetle) were observed by both growers and researchers to be associated with declining and dead trees experiencing sudden apple decline (O'Gorman 2020; JLM, unpublished data). Sudden apple decline is described as a decline in vigour over months or years, followed by sudden, rapid mortality over 2–3 weeks, with associated signs of decline including wilting foliage and cracking and flaking bark. Typically, a necrotic canker near the graft union has been associated with the collapse (O'Gorman *et al.* 2020).

Previous research has suggested a relationship between necrotic tissue and increased *S. myopaeformis* infestation on apple trees in Eurasia (Strapazzon and Granata 1984). The fungal pathogen *Cytospora* sp., which was the primary isolate from 2018 fungal surveys (but not subsequent years) of sudden apple decline canker (O'Gorman *et al.* 2020), has also been linked to improved neonate survival and increased larval densities (Bolay *et al.* 1976).

Producers' concerns around sudden apple decline, and the disease's links to necrotic cankers and *S. myopaeformis*, have led to speculation of an association between the two. This study investigates the hypothesis that a relationship between *S. mypaeformis* infestations and necrotic tissue (cankers) exists in apple trees in the Okanagan and Similkameen Valleys, British Columbia.

MATERIALS AND METHODS

Surveys of 50 sequential trees within orchard blocks, from each of 10 apple orchards (total: n = 500), were assessed for presence or absence (index of 1 or 0) of signs of *S. myopaeformis* infestation (frass, pupal exit holes, pupal casings) and presence or absence of necrotic cankers. Trees were visually assessed in a non-intrusive and non-destructive manner. A Chi-square test of independence was performed to assess a relationship between cankered/non-cankered trees and presence of *S. myopaeformis* in SAS® Studio 3.81, Basic Edition (2012–2020, SAS Institute Inc., Cary, North Carolina, United States of America). Nineteen trees that were assessed as "dead" among the 500 were excluded from this analysis (total: n = 481).

A subsequent survey investigated the association between *S. myopaeformis* and the necrotic cankers. Fifty trees from three orchards (total: n = 150) were surveyed for presence of necrotic cankers within the area 15 cm above each tree's graft union, and maximum necrotic canker length and width (cm) and number of signs of *S. myopaeformis* from both cankered and non-cankered tissue were recorded. The area above the graft union (scion) was isolated for assessment because tree tissue below the graft union generally had confounding qualities, such as mechanical damage, burr knots, suckering wounds, etc., whereas the tissues above the graft union typically did not.

Necrotic canker area was estimated by the simple formula: *maximum width* (cm) × *maximum length* (cm) = *estimated necrotic area* (cm^2). Estimated necrotic canker area per tree was plotted against average counts of *S. myopaeformis* signs (frass, pupal exit holes, pupal casings) per tree for each orchard (Microsoft® Excel® for Microsoft 365 MSO, Version 2307, Build 16.0.16626.20198; Microsoft Co., Redmond, Washington, United States of America) and the Spearman correlation coefficient value was generated (SAS®

Studio 3.81 Basic Edition, 2012–2020, SAS Institute Inc.). The average number of *S. myopaeformis* signs counted within necrotic areas *versus* non-necrotic areas per individual tree was analysed with a Wilcoxon signed rank test (SAS® Studio 3.81, Basic Edition, 2012–2020, SAS Institute Inc.).

Representative samples of larvae and pupae were collected from declined (dead) trees through excavation and stored in 95% EtOH. Samples were sent to the Canadian National Collection of Insects, Arachnids and Nematodes – National Identification Service (Central Experimental Farm, 960 Carling Avenue, Ottawa, Ontario, Canada) for identification and to be retained as voucher material.

RESULTS AND DISCUSSION

The Chi-square test of independence of surveyed trees suggests a significant relationship exists between presence of necrotic cankers and evidence of *S. myopaeformis* infestation ($X^2(1, N = 481) = 108.3869$, P = <.0001; Fig. 1). This supports the hypothesis that an association exists between the presence of necrotic cankers and the presence of *S. myopaeformis* in a tree.

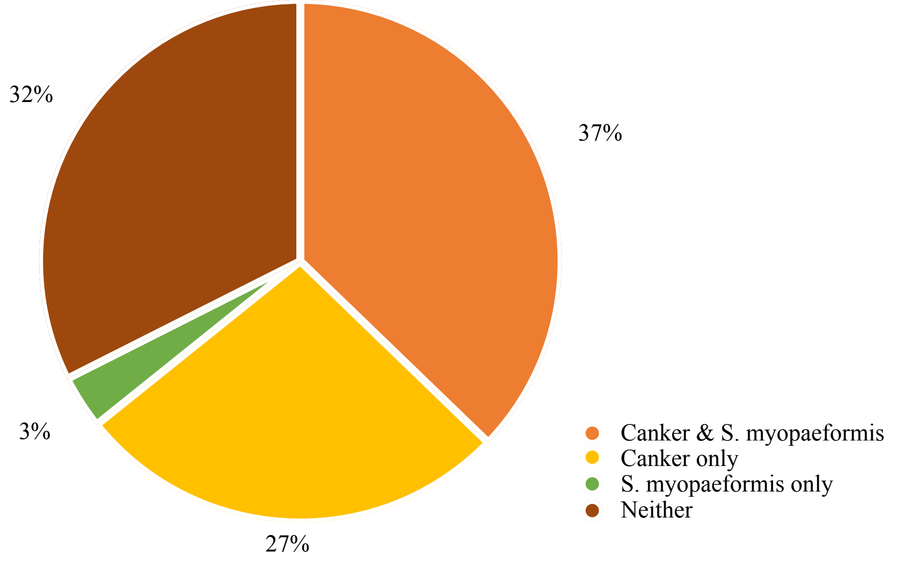

Figure 1. Proportion of trees from blocks of 50 trees from 10 *Malus domestica* orchards (19 dead trees removed from analysis; total: $n = 481$) in the Similkameen and Okanagan Valleys, British Columbia, comparing those with necrotic canker and no necrotic canker to those with signs of *Synanthedon myopaeformis* and no signs of *S. myopaeformis* infestation. A Chi-square test of independence was performed to assess the relationship between cankered/non-cankered trees and presence of *S. myopaeformis*: it showed a significant relationship between the presence of necrotic cankers and evidence of *S. myopaeformis* infestation ($X^2(1, N = 481) = 108.3869$, P = < 0.0001).

The Wilcoxon signed rank test showed a significant difference between cankered *versus* non-cankered tissue above the graft union exhibiting signs of *S. myopaeformis* infestation ($P < 0.0001$); all infestation signs (100%) were observed within cankered tissue in the scion wood surveyed, whereas none (0%) were observed in non-cankered tissue (Fig. 2).

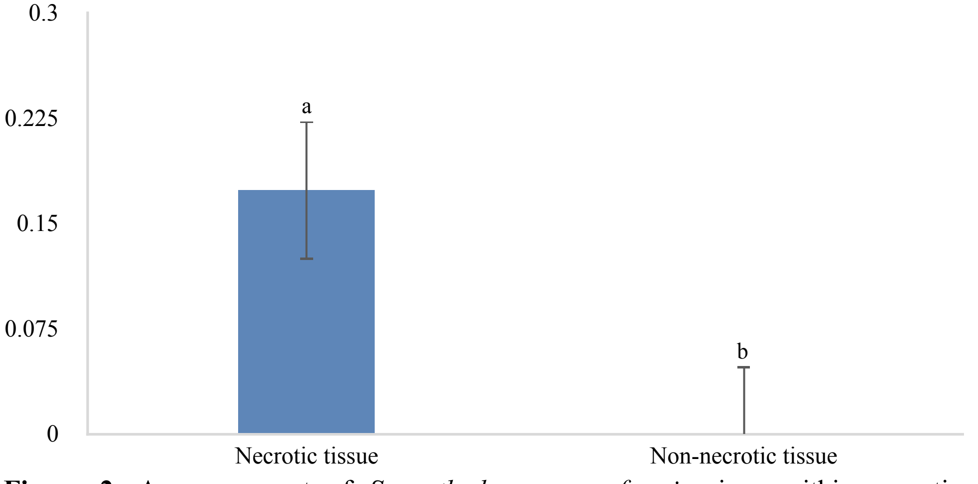

Figure 2. Average count of *Synanthedon myopaeformis* signs within necrotic (cankered) tissue *versus* non-necrotic (non-cankered) tissue within 15 cm above the graft union from 150 *Malus domestica* trees in the Okanagan Valley, British Columbia. Data were analysed with a Wilcoxon signed rank test. Different letters denote statistically significant differences ($P < 0.0001$); standard error bars are shown. One hundred per cent (100%) of the evidence of *S. myopaeformis* infestation was recorded in cankered area. It is important to note that signs of *S. myopaeformis* were noted in other areas of the tree (*e.g.,* Burr knots, pruning wounds) outside of this surveyed area; the studied region of the tree was targeted because it was typically only necrotic and non-necrotic tissue present and other confounding factors were minimal in this area of the tree.

It should be noted, however, that *S. myopaeformis* infestation was observed in some cases outside the investigated area (e.g., below the graft union) in otherwise vulnerable but non-cankered areas of the rootstock (*e.g*., burr knots); these regions were not assessed due to difficulty in accurately differentiating between and measuring cankered and non-cankered tissue there. One orchard exhibited necrotic cankers but had no *S. myopaeformis* infestations within the surveyed trees; however, this orchard was known to have had *S. myopaeformis* presence in past seasons (JLM, unpublished data). It is possible that an older, currently undetectable, infestation is previously associated with those cankers; however, it is unclear how long evidence of infestation is detectable. It is also possible that previous entry and exit holes enabled subsequent successful fungal entry through the wounds. Although this study adds to the evidence of an association between *S. myopaeformis* and necrotic cankers in apple trees, it does not help elucidate which is enabling which: is moth infestation promoting canker development, or is canker development enabling moth entry into the trees?

A significant positive relationship was found between necrotic canker size and count of *S. myopaeformis* signs (Spearman's correlation coefficient, *r*(N =

150) = 0.48807, *P* = <.0001; Fig. 3). An orchard with no *S. myopaeformis* sign that was observed within the survey area also exhibited the smallest average canker size and smallest average tree girth amongst trees, on average (data not shown). The estimated size of necrotic area (*maximum width* (cm) × *maximum length* (cm) = *area* (cm^2)) is only a general approximation of canker size and should be recognised only as such. A more comprehensive study would be beneficial in the future.

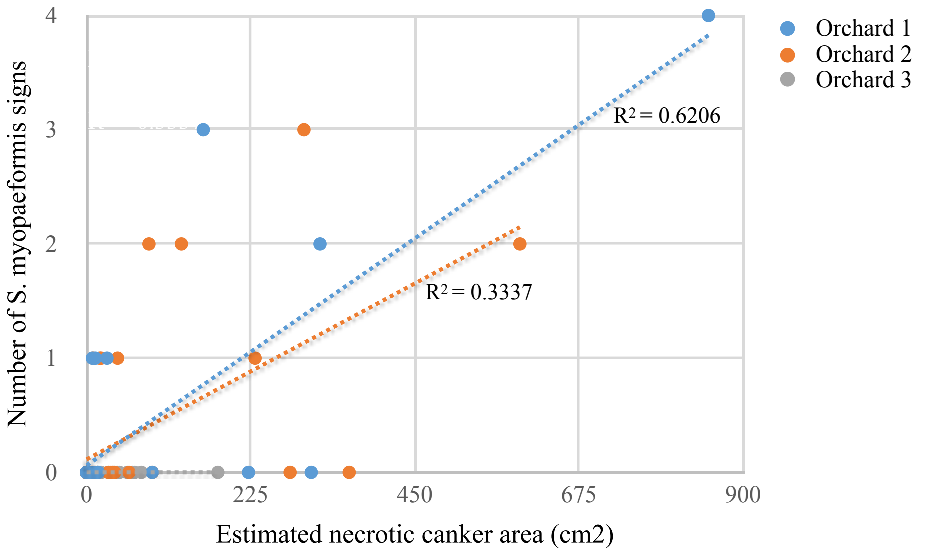

Figure 3. Number of *Synanthedon myopaeformis* signs (pupal casings, frass, exit holes) within necrotic (cankered) areas plotted against approximate area of cankered tissue assessed from 50 trees, repeated through the three orchards (total: *n* = 150), from observations made within 15 cm above *Malus domestica* graft unions in Okanagan and Similkameen valley, British Columbia, orchards. Trees without cankers were excluded. Regression values of individual orchards were generated. Pooled data showed a significant positive relationship between necrotic canker size and count of *S. myopaeformis* signs (Spearman's correlation coefficient, *r*(N = 150) = 0.48807, *P* = < 0.0001).

It is generally accepted that *S. myopaeformis* is more likely to infest damaged tree tissues (burr knots, mechanical damage, flaking bark) than healthy tissues, similar to *S. scitula* (Harris) (Bergh and Leskey 2003); necrotic tissue also appears to fulfill this role. *Synanthedon myopaeformis* populations have been increasing in the study region in British Columbia, correlating with producers' growing concerns over necrotic stem cankers on apple trees. Management of cankers may be an important aspect of a pest management plan for *S. myopaeformis*. Further work is needed to understand the relationship between *S. myopaeformis* and canker-causing fungal pathogens, especially *Cytospora* sp.; Aurelian (2011) suggests a potential symbiotic relationship.

Voucher specimens of larvae and pupae retained at the Canadian National Collection of Insects, Arachnids and Nematodes (NIS 2020-036) were confirmed as *Synanthedon* sp. and probable *S. myopaeformis*, based on taxonomy and host plant association, but further molecular confirmation was not conducted.

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