A molecular and genomic reference system for conifer defence against insects

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Abstract
Insect pests are part of natural forest ecosystems contributing to forest rejuvenation but can also cause ecological disturbance and economic losses that are expected to increase with climate change. The white pine or spruce weevil (Pissodes strobi) is a pest of conifer forests in North America. Weevil–host interactions with various spruce (Picea) species have been explored as a genomic and molecular reference system for conifer defence against insects. Interactions occur in two major phases of the insect life cycle. In the exophase, adult weevils are free-moving and display behaviour of host selection for oviposition that is affected by host traits. In the endophase, insects live within the host where mobility and development from eggs to young adults are affected by a complex system of host defences. Genetic resistance exists in several spruce species and involves synergism of constitutive and induced chemical and physical defences that comprise the conifer defence syndrome. Here, we review conifer defences that disrupt the weevil life cycle and mechanisms by which trees resist weevil attack. We highlight molecular and genomic aspects and a possible role for the weevil microbiome. Knowledge of this conifer defence system is supporting forest health strategies and tree breeding for insect resistance.

KEYWORDS
adaptation, bark beetle, chemical defence and physical defence, forest health, Pissodes strobi, plant insect interaction, plant resistance, stone cells (sclereids), terpene oleoresin, white pine weevil or spruce weevil

1 | INTRODUCTION

Conifers, such as spruces (Picea spp.) and pines (Pinus spp.), are some of the longest living plants and dominate some of the world’s largest forest ecosystems. Conifer forest health is essential for a sustainable balance of global carbon cycles and terrestrial biodiversity. Conifers are exposed to insect pests, which are natural components of forest ecosystems that contribute to forest rejuvenation. However, under certain conditions, forest insect pests can cause major ecological disturbance and economic losses. A recent example that highlighted the massive ecological and economic impacts of a forest insect pest is the mountain pine beetle (Dendroctonus ponderosae) epidemic. This insect outbreak has affected over 45 million hectares of pine forests in western North America and was attributed, at least in part, to anthropogenic factors such as long-term fire suppression and climate change (Safranyik & Carroll, 2006; Sambaraju, Carroll, & Aukema, 2019; Raffa et al., 2008; Raffa, Powell, & Townsend, 2013). Here, we focus on a major insect pest of spruce forests, the weevil Pissodes
strobi, and its interactions with resistant and susceptible hosts. This system has been developed as a reference for understanding defence and resistance mechanisms of conifers, including the molecular and genomic underpinnings of host resistance. Insights from the spruce-weevil system are broadly relevant to other conifer pests interactions. For select comparisons, we also refer to spruce interactions with other pests such as the bark beetles or budworms.

P. strobi is a beetle (Coleoptera) found throughout North America (Figure 1) and a member of the family Curculionidae. In addition to weevils, several of the most devastating forest insect pests worldwide belong to this family, including bark beetles (subfamily Scolytinae) and ambrosia beetles (subfamily Platypodinae). P. strobi was previously considered three distinct species due to its wide geographic distribution and multiple host species but is now generally recognized as a single species (Laffin, Langor, & Sperling, 2004; Langor & Sperling, 1995, 1997; Phillips & Lanier, 2000; Smith & Sugden, 1969). P. strobi is referred to in the literature with several common names, which differ in relation to geographic origin and host association (Laffin et al., 2004). For instance, in eastern North America, P. strobi attacks primarily eastern white pine (Pinus strobus) and is referred to as white pine weevil. In western North America, it attacks mainly spruce species and is called the spruce weevil. In this review, which is mostly based on research on ecological, biochemical, and molecular interactions with spruce, we refer to P. strobi as spruce weevil.

![Spruce weevil and spruce host distribution in North America](Image)

**FIGURE 1** Spruce weevil and spruce host distribution in North America. Distribution of spruce weevil (purple) throughout North America in relation to endemic spruce hosts white (light blue), Engelmann (grey), and Sitka (green) spruce (Source: weevil, Natural Resources Canada, Canadian Forest Service; spruce, USGS, USA). Geographic origin of important Sitka (Q903, H898), hybrid white (PG29), and white (WS77111) spruce genotypes are highlighted.

The spruce weevil is the most significant pest of regenerating spruce forests in North America (Ebata, 1991). Native North American spruce species affected by the weevil include Sitka spruce (Picea sitchensis), white spruce (Picea glauca), Engelmann spruce (Picea engelmannii), and hybrid spruce (P. glauca × engelmannii × sitchensis). These species comprise a genetic admixture with active gene flow across groups (De La Torre, Wang, Jaquish, & Aitken, 2014). Norway spruce (Picea abies) is affected in planted forests in eastern North America. Sitka spruce is particularly susceptible to weevil attack, which has led to several decades of low reforestation with this species in its native range in the Pacific Northwest despite its excellent fibre and wood quality (King & Alfaro, 2009). Weevil-resistant hybrid spruce (Kiss & Yanchuk, 1991) and Sitka spruce (King & Alfaro, 2009; King, Alfaro, Lopez, & Akker, 2011) genotypes have been discovered and are being used in tree breeding programmes that focus on forest health. The identification of resistant genotypes has served as a unique foundation to unravel the complex weevil–host interactions on the genomic, molecular, biochemical, and mechanistic levels (Byun-McKay et al., 2003; Byun-McKay et al., 2006; Hamberger, Ohnishi, Hamberger, Séguin, & Bohlmann, 2011; Keeling & Bohlmann, 2006; King, Alfaro, & Cartwright, 2004; King, Yanchuk, Kiss, & Alfaro, 1997; Kiss & Yanchuk, 1991; Miller, Madilao, Ralph, & Bohlmann, 2005; Moreira, Alfaro, & King, 2012; Nault, Manville, & Sahota, 1999; Porth et al., 2012; Porth, Hamberger, White, & Ritland, 2011; Porth, White, Jaquish, & Ritland, 2018; Ralph, Yueh, et al. 2006; Robert et al., 2010; Robert & Bohlmann, 2010; Whitehill et al., 2016; Whitehill et al., 2019; Whitehill, Henderson, Strong, Jaquish, & Bohlmann, 2016).

Here, we review known traits associated with spruce resistance and highlight how multiple defences are effective against the weevil at various stages throughout its life cycle. Additionally, we highlight observations that require further exploration but are hypothesized to play roles in the arms race between weevil and spruce. Knowledge of effective spruce defence mechanisms and their underlying genomics and biochemistry are supporting the development of pest management and forest health strategies to reduce the ecological and economic impact of this pest. This includes the development of methods for rapid phenotyping and genotyping of weevil resistance in spruce breeding programmes.

2 THE SPRUCE WEEVIL LIFE CYCLE DEFINES THE EXOPHASE AND ENDOPHASE OF WEEVIL–HOST INTERACTIONS

Weevil–host interactions can be separated into two major phases, which we are introducing here as the exophase and endophase. These two phases are primarily defined by different stages of the weevil life cycle (Figure 2).

In the exophase, the adult weevil is outside of the tree, its mobility is not restricted by host defences, and the insect can make choices between different trees to select hosts on which to feed and oviposit. Host choice for oviposition in the exophase ultimately affects
offspring survival and reproductive success. Adults overwinter in leaf litter at the base of or near host trees. In the spring, adult weevils climb or fly to the apical shoot tip of young trees, where they feed (maturation feeding) and mate. Adult feeding, which is restricted to small feeding punctures into the cortex and phloem, does relatively little damage to the host tree and is not typically a cause for tree mortality. Female weevils deposit eggs into oviposition holes along sterigmatal ridges of previous year apical shoot (PYAS) tips and cover eggs with a faecal cap composed of weevil frass and host oleoresin. Following oviposition, adults continue to feed throughout the growing season until returning to the forest floor in the fall to overwinter and repeat the cycle (Silver, 1968; Turnquist & Alfaro, 1996). Weevils are exposed to host defences during maturation feeding and oviposition. The behaviour and choices of female weevils to avoid or reduce the impact of host defences prior to oviposition increase offspring survival during the development from egg to emerging adults. The exophase is critical for selecting an optimal host for oviposition and reproductive success.

The endophase includes the developing insect from egg through larvae and pupa to young adult and occurs inside of the host cortex. During the endophase, mobility of the weevil is restricted, it can no longer make host choices, and it is affected by host defences without the option of escape. The developing weevil in the endophase causes significant damage to susceptible hosts as a result of feeding on the cortex, phloem, cambium, and outer xylem of the PYAS, which affects apical tree growth. Because weevils typically select young host trees, the endophase can cause tree mortality if the same tree is attacked repeatedly over multiple years. Mortality of weevil damaged trees is increased by competing vegetation, which further suppresses growth.

**FIGURE 2**  Spruce weevil life cycle in North America. The endophase (inner circle) is characterized by the egg, larvae, and pupae stages that occur inside a host tree. The exophase (outer circle) is defined by the adult weevil that lives primarily outside a host tree. Letters (J–M) indicate the month of the annual life cycle [Colour figure can be viewed at wileyonlinelibrary.com]

**FIGURE 3**  Typical distribution and abundance of spruce weevil eggs along previous year apical shoot (PYAS). (a) Diagram of current year apical shoot (CYAS) and PYAS with the three locations analysed for number of eggs oviposited. (b) Total number of eggs isolated from each location per PYAS (N = 17). Error bars represent the standard error of the mean. Differences between locations were analysed using one-way analysis of variance. Different letters indicate significantly different means separated post hoc following significant F tests by the protected LSD test (α = 0.05; Whitehill et al., 2019) [Colour figure can be viewed at wileyonlinelibrary.com]
Oviposition occurs in the spring and early summer before or around the time of apical bud burst, mostly within the top 5 to 10 cm of the PYAS below the apical bud. In a survey of naturally attacked hybrid spruce (Whitehill et al., 2019), we found oviposition in the top 1 to 10 cm [6.3 cm (±5.7 cm)] of PYAS, with most (86%) of the eggs in the top 4 cm (Figure 3). Eggs take 7 to 10 days for eclosion (Harman, 1970; Silver, 1968; Whitehill, Henderson, Strong, et al., 2016). Neonate larvae emerge from eggs, orient down the apical shoot, and feed on the nutrient-rich cortex, phloem, cambium, and outer xylem (Manville, Sahota, Hollmann, & Ibaraki, 2002). Larvae progress through four instars prior to excavating pupal chambers in the xylem, also called chip cocoons, which are composed of xylem material compacted with oleoresin (Harman, 1970; Whitehill et al., 2019; Whitehill, Henderson, Strong, et al., 2016). Larvae feeding destroys the PYAS phloem and xylem, which transport water and nutrients to the expanding current-year apical shoot, as well as the cambium, which is essential for radial growth. Thus, on a susceptible host, successful development of weevils in the endophase typically kills both the current-year apical shoot and PYAS, resulting in the loss of 2 years of apical growth on a young tree (Gara & Wood, 1989). Adult weevils exit the host by chewing emergence holes. Newly emerged adults remain on the host to feed until moving to the leaf litter in the fall to overwinter.

Over the complete life cycle of the exophase and endophase, larvae are the most devastating life stage. Weevil larvae are adapted to survive under the extreme conditions affected by the complex conifer defense system. Spruce defenses that provide durable weevil resistance are those that prevent or reduce oviposition and larval development.

3 | SPRUCE GENETIC AND GENOMIC RESOURCES TO STUDY WEEVIL RESISTANCE

Identification of genetic resistance, and its utilization in spruce breeding programs, is considered the best long-term approach to manage spruce weevil and support spruce reforestation and plantation in areas impacted by the weevil (King & Alfaro, 2009). Weevil-resistant spruce was first identified in the 1940s. Resistance has been observed in different spruce species and populations throughout the endemic range of the insect. For instance, weevil-resistant Sitka spruce was identified in three geographically distinct populations, and resistance was attributed to different defense traits (King et al., 2011; Moreira et al., 2012). Multiple populations of weevil-resistant hybrid spruce have also been identified throughout the hybridization zone of this species (Alfaro, van Akker, Jaquish, & King, 2004). Due to introgression of spruce species in the geographic range of the weevil, resistance traits and their underlying genes may be passed between species. Spruce genotypes from weevil-resistant populations have been selected based on high levels of durable resistance under conditions of extreme weevil pressure from natural or artificially augmented infestations (Alfaro et al., 2004; King et al., 2011; Kiss & Yanchuk, 1991). Some of these genotypes have been used to dissect defense mechanisms against weevils.

Spruce defenses that are relevant for weevil resistance are diverse and affect multiple facets of the weevil life cycle. Much of the knowledge of these defenses is based on comparative ecological, biochemical, molecular, and genomic studies with two naturally occurring Sitka spruce genotypes that represent contrasting weevil-resistant and susceptible phenotypes (Byun-McKay et al., 2003; Byun-McKay et al., 2006; Hall et al., 2011; Robert & Bohlmann, 2010; Robert et al., 2010; Whitehill et al., 2019; Whitehill, Henderson, Schuetz, et al., 2016; Whitehill, Henderson, Strong, et al., 2016). The highly weevil-resistant Sitka spruce genotype H898 used in those studies originates from an area in the Pacific Northwest (Figure 1) where high weevil population densities have naturally occurred, which may have driven the evolution of natural resistance (King et al., 2011). An extremely susceptible genotype, Sitka spruce Q903, was selected from the weevil-free Haida Gwaii Islands off the west coast of Canada, from a population that lacks a shared evolutionary history with the weevil. Both genotypes have been clonally propagated. The comparative studies with H898 and Q903 have been enabled, in part, by genomic insights in other Sitka spruce genotypes and other spruce species (Birol et al., 2013; Celenord et al., 2017; Nystedt et al., 2013; Ralph et al., 2008; Warren et al., 2015; Whitehill et al., 2019). Genomic resources have been developed using the highly weevil-resistant hybrid spruce genotype PG29 and the white spruce genotype WS77111, which is assumed to be weevil resistant (Bouillé, Senneville, & Bousquet, 2011; Jackman et al., 2016; Pavy et al., 2017; Warren et al., 2015). Other resources include quantitative and qualitative genetic and genomic information and knowledge of weevil resistance inheritance from spruce progeny trials (Alfaro et al., 2004; King et al., 2004; Kiss & Yanchuk, 1991; Porth et al., 2011; Porth et al., 2018; Verne, Jaquish, White, Ritland, & Ritland, 2011).

4 | SPRUCE DEFENCE SYSTEMS

The spruce defence systems function through synergism of chemical and physical traits; some of which are constitutively present or induced by weevil feeding or oviposition (Figure 4). Many of the spruce defenses that affect weevils are also effective as general conifer defenses against other herbivores (Franceschi, Krokene, Christiansen, & Krekling, 2005; Keeling & Bohlmann, 2006; Schiebe et al., 2012). However, certain defenses may have evolved to become particularly prominent in the spruce—weevil system, such as the induced formation of traumatic resin ducts (TRDs; Byun-McKay et al., 2003; Christiansen et al., 1999; Hudgins, Christiansen, & Franceschi, 2003; Hudgins & Franceschi, 2004; Krekling, Franceschi, Krokene, & Solheim, 2004; Martin, Tholl, Gershenzon, & Bohlmann, 2002; Nagy, Franceschi, Solheim, Krekling, & Christiansen, 2000; Zulak & Bohlmann, 2010), the terpene metabolites (+)-3-carene and dehydroabiatic acid (Hall et al., 2011; Hamberger et al., 2011; Roach, Hall, Zerbe, & Bohlmann, 2014; Robert et al., 2010; Whitehill et al., 2019), or an abundance of stone cells (Whitehill et al., 2019; Whitehill, Henderson, Schuetz, et al., 2016; Whitehill, Henderson, Strong, et al., 2016).
Chemical and physical defences of spruce against weevil have been characterized on the genomic, molecular, and biochemical levels; at physical scales ranging from molecules, cell types, tissues, and individual trees to populations; and across several different spruce species. A major chemical and physical defence system of spruce is the terpene rich oleoresin (Franceschi et al., 2005; Keeling & Bohlmann, 2006). Oleoresin consists of many (several hundred) different monoterpenes, sesquiterpenes, and diterpenes, the latter mostly in the form of diterpene resin acids (DRAs). The physical impact of resin on weevils in the exophase and endophase may be mostly attributed to the abundance, viscosity, and lipophilic nature of terpenes (Hanover, 1975). The physical effects of resin may not be dependent on the particular composition of resin beyond the general proportions of monoterpenes and sesquiterpenes relative to DRAs. However, individual resin chemicals, specifically the monoterpenes (+)-3-carene and the DRA dehydroabietic acid, have been associated with weevil resistance in Sitka spruce (Hall et al., 2011; Robert et al., 2010). Ecological roles for individual terpene metabolites remain to be explored, but evidence does point to potential roles for some compounds as juvenile hormone mimics, which could disrupt insect growth and development (Bohlmann, Crock, Jetter, & Croteau, 1998; Oh et al., 2017).

In stems of young spruce trees, oleoresin is constitutively present mostly in axial resin ducts in the cortex (cortical resin ducts, CRDs) and in radial ducts (Zulak & Bohlmann, 2010; Figure 4). In addition, oleoresin biosynthesis is induced in response to wounding (Byun-McKay et al., 2003), insect attack (Miller et al., 2005; Ralph, Yuen, et al., 2006), or treatment with the elicitor molecule methyl jasmonate (Celedon et al., 2017; Franceshi, Krekling, & Christiansen, 2002; Hudgins & Franceschi, 2004; Martin et al., 2002; Zulak et al., 2009). Induced oleoresin biosynthesis occurs in CRD and in newly formed TRDs that are initiated in the cambium zone and become apparent as concentric rings in the developing xylem (Christiansen et al., 1999; Martin et al., 2002; Zulak & Bohlmann, 2010).

Another well-established spruce chemical defence system consists of phenolic compounds; some of which are associated with polyphenolic parenchyma cells (Franceschi et al., 2005; Franceschi, Krekling, Berryman, & Christiansen, 1998; Jyske et al., 2016; Li et al., 2012). Specific phenolic compounds like the proanthocyanidin polymers found in spruce bark inhibit growth of mutualistic fungi required by conifer killing bark beetles to successfully colonize a host tree (Hammerbacher et al., 2011; Hammerbacher et al., 2014). Transcript abundance of genes of proanthocyanidin biosynthesis responds to fungal inoculation (Hammerbacher et al., 2011; Hammerbacher et al., 2013; Hammerbacher et al., 2014). Although phenolics are important in the defence of spruce against bark beetles and their associated fungi (Hammerbacher et al., 2011; Hammerbacher et al., 2013; Hammerbacher et al., 2014), a role for phenolics in weevil defence has not yet been thoroughly investigated. Acetophenone phenolics are involved in spruce resistance against the foliage feeding spruce budworm (Mageroy et al., 2015; Mageroy et al., 2017; Parent, Giguère, Mageroy, Bohlmann, & MacKay, 2018) but are not known to affect stem feeding insects.

Stone cells (sclereids) are a physical trait that negatively impacts bark beetle and weevil life cycles in spruce (King et al., 2011; Krokene, 2016; Wainhouse, Cross, & Howell, 1990; Whitehill et al., 2019; Whitehill, Henderson, Schuetz, et al., 2016; Whitehill, Henderson, Strong, et al., 2016). In Sitka spruce, stone cells are important for weevil resistance in the reference genotype H898 (Whitehill et al., 2019). The spatial distribution of stone cells in the cortex is critical to its effectiveness against weevils in the endophase. For example, the weevil-resistant Sitka spruce H898 has high quantities of stone cells primarily within the top 0–2 cm of the PYAS, the preferred site of

**FIGURE 4** Anatomy, histology and cell-types of spruce defences systems of a schematic spruce previous year apical shoot (PYAS). Physical and chemical defence systems are spatially depicted within a cross-section diagram of a typical susceptible spruce genotype from the top 0–2 cm of a PYAS. Defence systems can also be temporally regulated following weevil attack and can be constitutive (*), induced (**), or both (**), which determines their impact on the weevil life cycle. An adult weevil is shown preparing to begin feeding at a sterigmatal ridge (yellow highlight). The abundance of individual defences varies greatly between spruce species and between genotypes within a species. Different tissue types are labelled in the PYAS. Abbreviations: CRD, cortical resin duct; Ob, outer bark; Ph, phloem; PP, polyphenolic; SCC, stone cell cluster; TRD, traumatic resin duct; VB, vascular bundle; X, xylem [Colour figure can be viewed at wileyonlinelibrary.com]
oviposition where stone cells directly interfere with feeding by neonate and developing larvae (Whitehill, Henderson, Schuetz, et al., 2016; Whitehill, Henderson, Strong, et al., 2016). It is also possible that adult females in the exophase probe for stone cells while feeding on PYAS, which may explain their choice to avoid resistant Sitka spruce H898 for oviposition (Robert & Bohlmann, 2010). In other spruce species, stone cells appear to be distributed with variable abundance throughout the cortex of the PYAS (Alfaro et al., 2004).

Stone cells and oleoresin terpenes may function synergistically in a complex defence syndrome that provides more durable resistance against weevils than a single-component alone (Whitehill et al., 2019). In the following sections, we highlight spruce defence traits with a known association, correlation, or mechanism involved in weevil resistance and how they impact the insect life cycle.

5 | EXOPHASE WEEVIL INTERACTIONS WITH SPRUCE

5.1 | Host selection, evasion behaviour, and effects on reproduction

The initial identification of weevil-resistant populations and genotypes of Sitka and hybrid spruce was based on apical shoot kill by weevils in replicated field trials (Alfaro & Ying, 1990; Kiss & Yanchuk, 1991; Ying, 1991). Subsequent analyses established associations, or correlations, of certain tree defences primarily affecting weevil resistance in the endophase. Weevil behaviour in the exophase to select or avoid trees for oviposition is not well understood as a factor contributing to resistance.

To evaluate the impact of host selection behaviour on the weevil life cycle and to identify factors that contribute to oviposition, choice and no-choice experiments using resistant and susceptible Sitka spruce were performed (Robert & Bohlmann, 2010). When given a choice between resistant and susceptible trees, females preferred to feed and oviposit on susceptible trees and avoided resistant trees. In no-choice experiments, females did feed on resistant trees, but ovary development was delayed and insects avoided oviposition on resistant trees (Robert & Bohlmann, 2010). Females did not produce viable offspring on resistant trees (Robert & Bohlmann, 2010; Sahota et al., 1998). These bioassays suggested adult weevils in the exophase distinguish between resistant and susceptible trees. Females may actively avoid reproducing on resistant trees by abandoning these trees while also delaying ovary development. The features by which females differentiate between resistant and susceptible trees are not known. We hypothesize that feeding adult weevils probe the quality of host tissues for presence of defence traits such as resin chemicals, stone cells, or nutritional attributes before they select or avoid a tree for oviposition based on host quality. Alternatively, specific compounds in the host tissue that remain to be discovered may directly affect physiology of ovary development, which would then lead to failure of oviposition (Robert & Bohlmann, 2010; Sahota et al., 1998).

It is important to note that both resistant and susceptible Sitka spruces express some of the same general conifer defences, such as constitutive and induced accumulation of oleoresin, suggesting that weevils are adapted to cope with those general defences. However, feeding adult females may be affected during host choice and reproduction by specific components of the host defence system in resistant trees including (a) the presence of specific oleoresin chemicals such as (+)-3-carene or dehydroabietic acid (Robert et al., 2010); (b) rapidly induced formation of TRD (Byun-Mckay et al., 2006; Robert & Bohlmann, 2010); (c) abundance of stone cells (Whitehill et al., 2019; Whitehill, Henderson, Schuetz, et al., 2016; Whitehill, Henderson, Strong, et al., 2016); (d) other specific chemicals or proteins resulting from weevil-induced gene expression (Ralph, Jancsik, & Bohlmann, 2007; Ralph, Park, Bohlmann, & Mansfield, 2006; Ralph, Yueh, et al., 2006; Whitehill et al., 2019); or (e) combinations and synergism between several of these factors (Whitehill et al., 2019). It has also been considered that terpene volatiles may affect weevil behaviour during host selection, but there is currently not sufficient evidence to support a role for host volatiles in weevil host choice (Robert & Bohlmann, 2010). With regard to a role of rapidly induced formation of TRD, it has been argued that resin ducts in the outer xylem may not affect adult weevils because their feeding is apparently restricted to the cortex and phloem (Manville et al., 2002). However, TRDs are part of a reticulate resin duct system connected through radial resin ducts with outer tissues. In the absence of experiments that have tested the three-dimensional contribution of TRDs to terpenes in the outer tissues in more detail, it is premature to dismiss their contribution as a factor affecting adult weevil physiology or behaviour.

Taken together, the current knowledge of adult weevil interactions with spruce provides support to the “mother knows best” hypothesis for weevil host selection in the exophase. This hypothesis states that female insects oviposit on hosts in which their progeny will best perform, which will ultimately optimize their own fitness (Bernay & Graham, 1988; García-Robledo & Horvitz, 2012). In the case of spruce weevil, adult females can actively evade suboptimal hosts for oviposition. Further research into oviposition preference should explore a diverse range of resistant and susceptible spruce genotypes throughout, and beyond, the endemic range to evaluate weevil host selection preference in relation to spruce defences and other cues.

5.2 | Active avoidance of host defences

Insects can deactivate or reduce the impact of host chemical defences through active mechanisms (Després, David, & Gallet, 2007), but this phenomenon has not been experimentally explored for weevils coping with spruce defences. On the basis of observations of maturation feeding prior to oviposition, we hypothesize that weevils could manipulate oleoresin defences as a means to improve conditions for progeny fitness through draining oleoresin at the site of oviposition. In planta, weevil larvae exposed to high levels of oleoresin showed reduced survival (Whitehill et al., 2019). Draining of oleoresin occurs as part of the adult weevil’s maturation feed prior to oviposition on PYAS, when
feeding punctures disrupt CRDs, which in turn may decrease the volume of resin affecting weevil eggs and larvae (Figure 5a). Reduction of oleoresin present in PYAS may increase survival of eggs and establishing neonate first instar larvae. These observations require further experiments to adequately test the hypothesis.

6 | ENDOPHASE WEEVIL INTERACTIONS WITH SPRUCE

6.1 | Weevil eggs and spruce defences

Eggs and neonate larvae are perhaps the two most vulnerable stages of the weevil life cycle. Eggs require 7–10 days for eclosion. During this time, eggs are susceptible to predators, parasitoids, and host defences (Alfaro & Borden, 1980; Harman & Wallace, 1971). Three predator or parasitoid species are commonly associated with spruce weevil, the predatory fly Lonchaea corticis, and the hymenopteran larval parasitoid Eurytoma pissaedis and Dolichomitus terebrans nubillipennis (McIntosh, 1997). However, it is unknown what role the tree plays in weevil–predator/parasitoid interactions. In other conifers, predators or parasitoids are attracted through volatile emissions induced by insect feeding (Mumm & Hilker, 2005). Weevil-induced release of terpene volatiles has been reported for Sitka spruce (Miller et al., 2005). The release of volatiles is not restricted to passive emission of terpenes from oleoresin secretions. Weevil induced-volatile emission in Sitka spruce also involves locally and systemically induced biosynthesis and diurnal emission of the monoterpenes and sesquiterpenes (-)-linalool, (+)-linalool, (E,E)-α-farnesene, and (Z)-α-bisabolol (Miller et al., 2005). Ecological experiments remain to be done to test the effect of terpene emissions from weevil-attacked Sitka spruce on the attraction of predators or parasitoids.

Weevils produce a large number of eggs to increase the chance of progeny success. However, high oviposition density can promote...
competition between progeny and increase mortality (McIntosh, 1997). A single female weevil can deposit between 75 to 350 eggs per PYAS (Belyea & Sullivan, 1956). Females cover oviposition sites with a faecal cap, which may protect eggs from predation. We are aware of only one study that showed spruce defence directly affecting egg survival on resistant white spruce (Alfaro, 1995), where eggs were flooded with resin originating from CRD and TRD resulting in egg mortality. Weevil eggs are exposed to oleoresin (Figure 5b), and high amounts of oleoresin encapsulating eggs may have chemical and physical effects that increase mortality.

6.2 | Neonate and early instar larvae interactions with stone cell defences

Arguably the most critical interaction between weevils and spruce occurs at the stage of freshly emerged larvae (neonate, first instar). The stone cell defence system of resistant hosts is highly effective in disrupting neonate establishment (Whitehill et al., 2019; Whitehill, Henderson, Strong, et al., 2016). The mechanisms of stone cells against larvae include (a) acting as a physical barrier, (b) causing mandle damage, and (c) physical displacement of nutritious host tissues. Neonate larvae cannot easily overcome these mechanisms of the physical stone cell defence. The physical constraints caused by stone cells appear to also enhance the impacts of oleoresin terpenes (Whitehill et al., 2019). The conclusion that stone cells are most effective at disrupting early establishment was based on in vitro bioassays, which used larvae on artificial diets containing stone cells isolated from Sitka spruce, and in planta experiments (Whitehill et al., 2019; Whitehill, Henderson, Strong, et al., 2016).

Stone cells are abundant in resistant Sitka spruce (Whitehill, Henderson, Schuetz, et al., 2016). They are physically tough, nutrient poor, and indigestible (Whitehill, Henderson, Strong, et al., 2016). Their high abundance delays or prevents instar progression and feeding through the cortex as observed by shorter feeding galleries on resistant trees (Whitehill et al., 2019), High abundance of stone cells impacts larvae growth, development, and physiology (Whitehill et al., 2019; Whitehill, Henderson, Strong, et al., 2016). Delayed development of larvae affected by stone cells also enhances the effects of stone cells on mandle wear in young larvae, as it prevents renewal of mandibles, which occurs during moult (Whitehill et al., 2019, Whitehill, Henderson, Strong, et al., 2016). Few larvae do progress to the third and fourth instar on resistant trees with high abundance of stone cells, but most larvae remain arrested in the early instar stages (Whitehill et al., 2019).

The combined antinutritive and physical effects of stone cells present a robust mechanism of defence against the weevil. There are no obvious strategies by which weevil larvae might adapt to stone cells in the endophase, unless they evolve mechanisms to digest the thick, lignin-rich cell walls of stone cells while also surmounting their physical impacts. The only obvious adaption is by adult females in the exophase avoiding oviposition on trees with high abundances of stone cell at the tip of the PYAS, which selects for this defence trait in spruce populations exposed to high weevil pressure.

6.3 | Late instar (third and fourth) larvae interactions with spruce defences

Once weevil larvae are established on a susceptible host and reach the third or fourth instar, intraspecies competition becomes a critical factor (Figure 5c; McIntosh, 1997). Of the large number of eggs placed by a female weevil on the PYAS of a susceptible tree, only a few dozen larvae will complete development and emerge as adults. On resistant Sitka spruce that was artificially inoculated with weevil eggs, very few larvae progress to the third and fourth instar or pupae (Whitehill et al., 2019). These larvae encounter increased quantities of terpene oleoresin as they feed (Whitehill et al., 2019; Figure 5d).

The effect of resin on the third and fourth instar larvae was seen in experiments using artificial egg inoculations at the tip of the PYAS of resistant and susceptible Sitka spruce (Whitehill et al., 2019). Larvae on resistant trees were encrusted in oleoresin, restricted from moving (Figure 5d), and their frass contained high quantities of terpenes, compared with frass from larvae on susceptible trees. On susceptible trees, larvae produced longer feeding galleries that were less impacted by oleoresin. The accumulation of host terpenes in the frass indicates that larvae on resistant trees were ingesting large amounts of oleoresin. Digestive tracts of some larvae were completely filled with oleoresin (Figure 5e).

When trees were artificially inoculated 4–6 cm below the area of highest density of stone cells, which is within the top 2 cm of the PYAS, larvae on both resistant and susceptible trees showed an unnatural acropetal, instead of the natural basipetal, movement (Figure 5e). In this scenario, larvae on both resistant and susceptible trees where heavily encrusted with oleoresin within feeding galleries and frass contained high amounts of host terpenes, suggesting that oleoresin acted as the major defence system. Differences in larval performance in this scenario may be attributed to variation in the composition of oleoresin between resistant and susceptible Sitka spruce. For example, resistant Sitka spruce H898 has higher quantities of the monoterpene (+)-3-carene and the DRA dehydroabiatic acid, compared with susceptible Q903 trees (Hall et al., 2011; Hamberger et al., 2011; Robert et al., 2010; Whitehill et al., 2019). Larvae may be exposed to potentially more toxic or otherwise more inhibitory components of oleoresin from resistant trees.

The combination of stone cells and oleoresin terpenes represents a synergistic “conifer defence syndrome” that functions through a combination of both “trapping” and “ambushing” weevils with physical and chemical defences (Whitehill et al., 2019). This defence syndrome of resistant trees provides a robust defence against weevil larvae (Whitehill et al., 2019).

6.4 | Weevil pupae interactions with spruce defences

Mature larvae produce chip cocoons in the outer xylem where they develop into pupae (Figure 5f). Pupae remain for several days to weeks in the chip cocoons prior to emerging from trees as adults. It
is not known that any of the described spruce defences act against pupae. Based on localization, it is possible that TRD and traumatic resin may affect pupae (Figure 6). However, given that it is the feeding larvae—not pupae—that inflict major damage to the host, any defence against pupae would likely be inconsequential for the tree.

7 | GENOMIC, MOLECULAR, AND BIOCHEMICAL UNDERPINNINGS OF SPRUCE DEFENSES AGAINST WEEVILS

Much work has been done to explore the genomic, molecular, and biochemical underpinnings of spruce oleoresin and terpene defences against weevils. This includes detailed analyses of constitutive and induced oleoresin terpene biosynthesis and accumulation in CRD and TRD, and characterization of TRD development. In addition, transcriptome- and genome-wide analyses have investigated spruce–weevil interactions more broadly. In contrast to oleoresin and terpene defences, nothing is known about the genomics and developmental biology of stone cells, the other major defence system against weevils. Using laser-capture microdissection and cell-type specific RNA-Seq, we have identified transcription factors and other genes that may contribute to stone cell formation based on their temporal and spatial patterns of expression (Whitehill & Bohlmann, unpublished results).

7.1 | Diversity and variation of terpenes, terpene synthases, and cytochromes P450

Terpene synthases (TPS) involved in the biosynthesis of the monoterpenes, sesquiterpenes, and diterpenes that comprise oleoresin have been identified and characterized in several conifer species. The topic of conifer TPS has been previously reviewed (Keeling & Bohlmann, 2006; Zerbe & Bohlmann, 2014), and subsequent progress on conifer TPS has been summarized in recent publications (Celedon et al., 2017; Shalev et al., 2018; Warren et al., 2015). Conifer TPS of secondary (i.e., specialized) metabolism, which includes oleoresin biosynthesis and terpene volatile emissions, belong to the gymnosperm-specific TPS-d subfamily of the plant TPS gene family (Chen, Tholl, Bohlmann, & Pichersky, 2011; Shalev et al., 2018; Warren et al., 2015). In contrast, conifer TPS involved in the primary (i.e., general) metabolism of gibberellin biosynthesis belong to the TPS-c and TPS-e/f subfamilies (Chen et al., 2011; Warren et al., 2015). The majority of known conifer TPS act on only three different isoprenyl diphosphate substrates to synthesize the dozens or hundreds of terpene secondary metabolites of conifer defence (Keeling & Bohlmann, 2006). Conifer monoterpenes (C_{10} molecules) are produced by monoterpen TPS syntheses (mono-TPS) from geranyl diphosphate, sesquiterpenes (C_{15} molecules) are produced by sesqui-TPS from farnesyl diphosphate, and diterpenes (C_{20} molecules) are produced by di-TPS from geranylgeranyl diphosphate. In addition, volatile hemiterpenes (C_{5} molecules) are produced by hemi-TPS from dimethylallyl diphosphate (Gray, Breneman, Topper, & Sharkey, 2011), and a few conifer di-TPS require copalyl diphosphate as a substrate (Hall et al., 2013; Keeling et al., 2010). The majority of terpenes of conifer secondary metabolism are linear or cyclic hydrocarbons, in addition to some hemiterpene, monoterpane, and sesquiterpene alcohols. These compounds are the products of TPS enzymes and are not typically further modified by other enzymes. In contrast, conifer diterpenes accumulate predominantly in the oxidized form of DRAs. DRA biosynthesis involves the combination of di-TPS and cytochromes P450 (P450s) of the gymnosperm-specific CYP720B family (Geisler, Jensen, Yuen, Madilao, & Bohlmann, 2016; Hamberger et al., 2011; Ro, Arimura, Lau, Piers, & Bohlmann, 2005). The different diterpene olefins, alcohols, aldehydes, and acids (i.e., DRAs) found in spruce oleoresin are generated through a combinatorial biosynthetic system of single- and multiproduct di-TPS combined with multisubstrate and multifunctional CYP720B enzymes (Geisler et al., 2016; Hamberger et al., 2011).

Analysis of the hybrid spruce (PG29), white spruce (WS7111), and Norway spruce genomes (Birol et al., 2013; Nystedt et al., 2013; Warren et al., 2015); the transcriptomes of white spruce, hybrid spruce, and Sitka spruce (Keeling, Madilao, Zerbe, Dullat, & Bohlmann, 2011; Celedon et al., 2017); and the Sitka spruce full-length cDNAs (Ralph et al., 2008) allowed for genome-wide analysis of the conifer TPS-d and CYP720B families and the pathways that produce the

FIGURE 6  Tissue damage inside the previous year apical shoot (PYAS) of Sitka spruce (susceptible genotype Q903) at successful completion of the endophase of the weevil life cycle. (a) Cross section of an excavated pupal chamber (PC) encompassing portions of the cortex and xylem. The formation of nascent traumatic resin ducts (TRD) line the outer edges of the xylem (shown with the outer yellow line). A few TRD are present in the inner ring (inner yellow line) of xylem suggesting response to a stimulus in the previous year. Close-up views of newly formed TRD can be seen in (b) and (c). Abbreviations: CRD, cortical resin duct; Fr, frass [Colour figure can be viewed at wileyonlinelibrary.com]
isoprenyl diphosphate precursors of conifer terpene diversity. The genome of the weevil-resistant hybrid spruce PG29 revealed 83 unique TPS genes (including 28 pseudogenes) and eight CYP720B genes among a total of 307 unique P450s (including 43 pseudogenes; Geisler et al., 2016; Warren et al., 2015). However, only seven of the TPS and none of the CYP720B genes of hybrid spruce PG29 have been functionally characterized (Keeling et al., 2010; Keeling, Madilao, et al., 2011; Zerbe, Chiang, & Bohlmann, 2012). For broader reference, functionally characterized TPS and CYP720B genes across different spruce species include approximately 30 mono-TPS genes (Byun-McKay et al., 2006, 2003; Füldt, Arimura, Gerszenzon, Takabayashi, & Bohlmann, 2003; Hall et al., 2011; Keeling, Madilao, et al., 2011; Martin, Füldt, & Bohlmann, 2004), fewer than 10 sesqui-TPS (Keeling, Madilao, et al., 2011; Martin et al., 2004), fewer than 10 di-TPS (Martin et al., 2004; Keeling et al., 2010; Keeling, Madilao, et al., 2011; Keeling et al., 2011), and only three CYP720B (Geisler et al., 2016; Hamberger et al., 2011). Thus, much more work is required to comprehensively identify the functions of terpene defence genes in the hybrid spruce PG29 genotype and in other spruce species for research on weevil resistance.

The structural diversity of spruce terpenes is the product of the multigene TPS-d and CYP720B gene families. Within the TPS gene family, even closely related genes often encode enzymes that produce different terpene profiles (Hall et al., 2011; Keeling, Madilao, et al., 2011; Keeling, Weisshaar, Lin, & Bohlmann, 2008; Roach et al., 2014). Many conifer TPS are multiproduct enzymes (Keeling, Madilao, et al., 2011; Keeling, Weisshaar, et al., 2011; Martin et al., 2004; Steele, Crock, Bohlmann, & Croteau, 1998), which further contributes to the terpene diversity in a given species. The functional diversity of conifer TPS genes appears to have originated through cascades of gene duplications with subsequent neo-functionalization or subfunctionalization (Keeling et al., 2008; Keeling, Madilao, et al., 2011; Martin et al., 2004; Warren et al., 2015). A well-characterized example of duplicated and functionally diverged TPS that are relevant for weevil resistance are the Sitka spruce mono-TPS genes responsible for the formation of the monoterpenes (+)-3-carene and (-)-sabinene, PsTPS-3car and PsTPSsab, respectively (Hall et al., 2011; Roach et al., 2014). PsTPS-3car produces mostly (+)-3-carene, and PsTPSsab produces predominantly (-)-sabinene. The enzymes encoded by these genes are over 82% identical, and as few as three pairwise amino acid substitutions are sufficient to interconvert product profiles between these two TPS (Roach et al., 2014). The weevil-resistant Sitka spruce genotype H898 has three PsTPS-3car gene copies, whereas the susceptible Sitka spruce genotype Q903 apparently has only two copies (Hall et al., 2011). Functional and enzyme kinetic characterization, as well as analysis of transcript and protein abundance, of the PsTPS-3car members in H898 and Q903 showed a presence (in H898)/absence (in Q903) variation of PsTPS-3car2, which explained the relatively high abundance of (+)-3-carene in H898, associated with weevil resistance, and the lack or low abundance of (+)-3-carene in Q903 (Hall et al., 2011; Robert et al., 2010). These results highlight how gene duplication and functional diversification, as well as presence/absence or copy number variation between two genotypes of the same species, can explain diversity of terpene metabolite profiles and their phenotypic variation associated with weevil resistance (Hall et al., 2011).

7.2 | Weevil-induced transcriptome and proteome changes

Weevil attack, or wounding and treatment with methyl jasmonate, which serves as a mimic for insect feeding, induces major changes of the spruce transcriptome and proteome (Celedon et al., 2017; Lippert et al., 2007; Ralph, Park, et al., 2006; Whitehill et al., 2019). This includes induced expression of TPS and CYP720B genes in bark and xylem (Byun-McKay et al., 2006, 2003; Celedon et al., 2017; Hall et al., 2011; Hamberger et al., 2011; Miller et al., 2005; Zulak et al., 2009). The induced transcriptome response is associated with activation of terpene biosynthesis and terpene accumulation in CRD and de novo terpene biosynthesis in newly formed TRD (Miller et al., 2005; Zulak et al., 2009; Zulak & Bohlmann, 2010; Zulak, Dullat, Keeling, Lippert, & Bohlmann, 2010). Spatially refined expression analyses of tissues dissected by laser-capture microdissection showed induced TPS and CYP450 gene expression in CRD and in the cambium zone that gives rise to TRD (Abbott, Hall, Hamberger, & Bohlmann, 2010; Hamberger et al., 2011). Coupling of laser-capture microdissection with large-scale RNA-Seq analysis revealed a dynamic, cell-type, and tissue-specific rearrangement of the white spruce transcriptome in CRD and phloem (Celedon et al., 2017). Within the complex bark tissue, expression of many genes of the terpenoid defence system is highly cell-type specific for CRD (Celedon et al., 2017). New work is now underway to dissect the induced transcriptome of the cambium zone and developing TRDs, which is revealing transcription factors that may be involved in the up-regulation of induced terpene defences (Celedon & Bohlmann, unpublished results).

7.3 | Ecological and genetic evidence for gene functions in weevil resistance

Ecological validation of functions of individual spruce genes or sets of genes involved in resistance against weevil is difficult to ascertain and would ideally require overexpression, RNA interference down-regulation, or CRISPR/CAS9-based gene editing. However, the development of transgenic spruce lines is not a trivial task and has only recently been achieved for a small set of spruce genes of terpenoid and phenolic biosynthesis (Hamberger et al., 2011; Hammerbacher et al., 2014; Hammerbacher et al., 2019; Schmidt et al., 2010). RNA interference of the DRA producing PsCYP720B4 enzyme in white spruce resulted in reduced levels of a few DRAs in white spruce (Hamberger et al., 2011), but the ecological effect of this downregulation has not yet been assessed. Although effects may be tested in the laboratory with controlled weevil feeding and oviposition experiments as described above, field tests with transgenic spruce are not possible under current legislation in Canada. There is also the problem of a large amount of functional redundancy between the different TPS
and CYP720B genes within the spruce defence system (Geisler et al., 2016; Hall et al., 2011; Hamberger et al., 2011; Keeling, Madilao, et al., 2011), which makes it nearly impossible to completely remove or selectively up- or down-regulate individual terpenes in the complex terpene metabolomes.

Recent emphasis has been placed on genetic associations of spruce with weevil resistance. Quantitative genetic approaches using individuals with known resistance phenotypes revealed associations between quantitative trait loci (QTLs) for weevil resistance with genes of the phenylpropanoid pathway but also concluded that weevil resistance is a complex trait that was not easily explained and requires more phenotypic information on resistance (Porth et al., 2011; Verne et al., 2011). Assessment of weevil behaviour and performance in the exophase and endophase, as described above, and new tools developed to assess variation of stone cells (Whitehill, Henderson, Schuetz, et al., 2016; Whitehill, Henderson, Strong, et al., 2016; Whitehill et al., 2019) and terpene profiles (Celedon et al., 2017; Hall et al., 2011) in the spruce defence syndrome will support the development of comprehensive phenotypic data. It will be critical to continue to integrate functional and quantitative approaches to identify the genetic components of weevil resistance. With advances of spruce genome assemblies (Warren et al., 2015), genomic selection for weevil resistance may also become feasible.

FIGURE 7 Model summarizing spruce–weevil interactions. (a) Diagrammatic representation of the exophase and endophase of the weevil life. The endophase (En; left side of previous year apical shoot [PYAS]) begins in early spring when an egg is oviposited at the top of a PYAS and ends when an adult emerges from the host in late summer, which begins the exophase (Ex). The exophase (right side of PYAS) is continuous for weevil adults year round. At time of host selection, adults may actively evade (AE) hosts after probing trees. Adults may also actively avoid (AA) spruce defence systems through draining oleoresin at feeding punctures to increase brood fitness. (b) Resistant (R) spruce effectively “blockade” larval progression through the bark with high quantities of stone cells (purple), which synergizes the impact of terpene oleoresin as it “ambushes” the weevil by filling shorter galleries to form a robust defence syndrome. Larvae that feed on susceptible (S) spruce complete the insect life cycle and feed further down the PYAS [Colour figure can be viewed at wileyonlinelibrary.com]}

and ambrosia beetles and their fungal and bacterial microbionts. Fungi and bacteria can be involved in the breakdown and metabolism of terpene and phenolic metabolites, and some of these host defence compounds can be exploited as a source of nutrients for microorganisms (Adams, Boone, Bohlmann, & Raffa, 2011; Boone et al., 2013; Hammerbacher et al., 2013; Wang et al., 2013, 2014). Until recently, the role of the microbiome has been largely overlooked in the interactions of weevils with their host trees (Berasatequi et al., 2016; Berasatequi et al., 2017; Whitehill et al., 2019). Work in the Hylobius abietis, Norway spruce system, showed that the weevil gut microbiome reduced the impact of conifer diterpenes and increased insect fitness (Berasatequi et al., 2017). A role for the microbiome has not yet been documented for the Pissodes strobi, spruce system, but structures on the weevil gut that house bacteria (bacteriomes) were substantially affected in larvae feeding on resistant Sitka spruce compared with susceptible Q903 trees (Whitehill et al., 2019). Bacteriomes are found in other weevil species and house bacteria that are part of an obligate symbiosis (Vigneron et al., 2014). Future work will determine if, and how, bacterial associations may contribute to the outcome of spruce–weevil interactions on resistant and susceptible trees.

8 | ROLE OF THE MICROBIOME IN SPRUCE–WEEVIL INTERACTIONS

Microbial associations are critical in many Curculionidae to overcome conifer defences and are perhaps best known for the tree-killing bark and ambrosia beetles and their fungal and bacterial microbionts. Fungi and bacteria can be involved in the breakdown and metabolism of terpene and phenolic metabolites, and some of these host defence compounds can be exploited as a source of nutrients for microorganisms (Adams, Boone, Bohlmann, & Raffa, 2011; Boone et al., 2013; Hammerbacher et al., 2013; Wang et al., 2013, 2014). Until recently, the role of the microbiome has been largely overlooked in the interactions of weevils with their host trees (Berasatequi et al., 2016; Berasatequi et al., 2017; Whitehill et al., 2019). Work in the Hylobius abietis, Norway spruce system, showed that the weevil gut microbiome reduced the impact of conifer diterpenes and increased insect fitness (Berasatequi et al., 2017). A role for the microbiome has not yet been documented for the Pissodes strobi, spruce system, but structures on the weevil gut that house bacteria (bacteriomes) were substantially affected in larvae feeding on resistant Sitka spruce compared with susceptible Q903 trees (Whitehill et al., 2019). Bacteriomes are found in other weevil species and house bacteria that are part of an obligate symbiosis (Vigneron et al., 2014). Future work will determine if, and how, bacterial associations may contribute to the outcome of spruce–weevil interactions on resistant and susceptible trees.

9 | CONCLUSION

This review summarizes much of the current knowledge of spruce–weevil interactions and provides a perspective on natural defence strategies that may be explored, or are already being explored, for breeding of spruce genotypes with durable resistance. Such breeding programmes for weevil resistance are ongoing for Sitka
spruce and hybrid spruce in western Canada by the British Columbia Ministry of Forests (full name: BC Ministry of Forests, Lands, Natural Resource Operations and Rural Development) and for Norway spruce in eastern Canada by J. D. Irving Limited. As highlighted here, interactions between the weevil and spruce throughout the weevil’s exophase and endophase are highly complex (Figure 7a). Durable resistance as described for individual genotypes of Sitka spruce and hybrid spruce is not based on a single defence mechanism but involves a multicomponent defence syndrome. Prominent components of the spruce defence syndrome are stone cells and constitutive and induced oleoresin, including specific terpenes such as (+)-3-carene. These components are often cell type or tissue specific in their expression. Thus, the specific temporal and spatial patterns with which the tree displays its defences have consequences for their impacts at the various stages of the weevil life cycle. For example, high density of stone cells at the tip of the PYAS affects the neonate weevils, whereas stone cells in the lower parts of the PYAS may be less effective against larvae in the later instars. The increased production of induced oleoresin, the biosynthesis of which is induced by weevils in cells of CRD and newly formed TRD, acts synergistically with stone cells against weevil larvae that are arrested in their movement (Figure 7b). These defences are now best characterized in the weevil-resistant Sitka spruce genotype H89B, and work is underway to characterize the defence and resistance mechanisms and underlying genomics and genetics in the weevil-resistant hybrid spruce genotype PG29. The integration of functional and quantitative genetic approaches will be critical to the elucidation of weevil resistance. Potential roles of the microbiome in the weevil–spruce interaction require further exploration as potential targets for pest management strategies.

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