

RNA Interference- A promising tool for forest pest management in this era of genomics

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Introduction

Due to their socio-economic and environmental functions, forests are extremely important. But internationally, a drop in coniferous woods is still unprecedented, as temperatures, dryness, wind and the plague grow. Most insect pest population breakouts rely on temperature and the frequency, intensity and cyclicity of forest pest outbursts are already well established as a result of climate based intensification. Furthermore, the geography of forest insects has extended by the exploitation of hitherto undiscovered natives because of low temperatures.

Such an extension in the field also leads to broad death of the trees, diminishing productivity of woodlands, and storage of carbon. As a result, catastrophic trees depletion owing to insect out branches or range expansions may generate pathways beyond forest ecosystems' resistance limitations leading to irreverent ecological changes.

RNAi is a strong technology which can introduce a new paradigm in the control of forest pesticides, although it still presents significant hurdles. In wood bored coleopterans scientists are developing a variety of RNAI boost methods, i.e., selection of appropriate target genes using data from sequence, genetic modification of microbes and plants, identification of extracellular vesicle components; dsRNA complexation/encapsulation with nanomaterials. In addition, the protection of forests using RNAi-based pesticides would also constitute a new integrated pest control method due to its high degree of specificity and safety compared to traditional pesticides. The researchers are therefore focused Researchers are therefore focused by this technique on managing forest pests and their potential to manage various coleopteran forest-boring pests, including Bark Beetles.

Wood-Boring Coleopterans

The bark beetles are the most serious and damaging conifer forest pesticides in the world (Coleoptera: Curculionidae: Scolytinae). As abiotic conditions are main drivers for population expansion of bark beetles, the frequency and severe outbreaks of these aggressive forestry pests owing to continuing global change are



likely to rise. Hot temperatures enhance the expansion of the bark beetle population by reducing winter mortality and developing time, enabling more generations each year. During the endemic phase of the forest ecosystem, most of the bark beetles reproduce on the weak and dead trees by recycling the nutrients from the dead plant tissue. However, once the bark beetle population increases to an epidemic level, they start attacking the healthy trees. Some aggressive bark beetles, such as the southern pine beetle (*Dendroctonus frontalis*) and the mountain pine beetle (*D. ponderosae*).

RNAi Based Forest Protection Products (FPPs) Against Wood-Boring Coleopteran

RNAi refers to a post-transcriptional gene silencing mechanism prohibiting protein formation by introducing environmental RNA. Three RNAi pathways have been characterized so far. These are common in insects but not in plants or other animals and include the small interfering RNA (siRNA) pathways, microRNA (miRNA) pathway, and piwiRNA (piRNA) pathway. However, siRNAs are highly sequence-specific to



target transcripts, and miRNAs are partial complementarity to target transcripts. In contrast to siRNAs and miRNAs, the piRNAs pathway is likely less understood

RNA interference (RNAi) is an evolutionarily conserved post-transcriptional gene silencing mechanism, which is triggered by exogenous double-stranded RNA (dsRNA). Thus, RNAi becomes a promising tool for forest pest management in this era of genomics.

Recent advancements in sequencing technology and platforms lead to higher availability of coleopteran forest pest genomes and transcriptomes that can serve as valuable resources for species-specific dsRNA design. It was pretty well known that coleopteran insects are usually susceptible to RNAi. Recently, reported the underlying cause of coleopteran insect susceptibility toward RNAi. With intriguing evidence of RNAi susceptibility in coleopterans, researchers started exploring the potential of RNAi in managing coleopteran forest pests. Nanoparticle encapsulation is proven highly effective in controlling many insects and may be used against forest pests via GMO-free approaches such as trunk injection or root absorption methods. Recent discoveries also indicated possibilities for a nano carrier-mediated transdermal dsRNA delivery system to enhance RNAi efficiency after spraying.

RNAi Mechanism in Insect Pests

It is a well-known phenomenon that the symbiotic blue-fine fungus paves the way for the colonisation by acting as the source of semi-chemical bark beetles, spruce-free chemical defence and nutrient supplements. It is a well-known phenomenon. Fungi also benefit from the inoculation of bark beetles into the phloem, as



they are not able to penetrate bark alone. The conclusion is that conifer bark beetles and their symbiont cause large forest mortality in Norway. That is why scientists should work concurrently using the RNAi tools to regulate symbionts and bark beetles. Foliar use of RNAi-based insecticides cannot be applied for the control of insect pests from wood because of the size of the trees and thick bark on the surface. By means of the host delivery dsRNA pesticides such as injecting trunk, soil draining, symbiotic plant and target pest microorganisms and viruses, the long-lasting protection of trees against insect pests and infections may be promised. With the RNAi-based FPPs it would be difficult to limit off-target and non-target effects. However the efficacy of this technique must be determined by species specific and target-specific RNAi targets. In the search for HDRs in the objective pest a bioinformatics pipeline may be beneficial. For such tactics, however, forestry genome sequences will be a requirement. For the future, research efforts are thus essential in order to

achieve superior species-specific goals for dsRNA applications in the areas of forest insect genomes and tissue-specific transcriptomes.

RNAi for Disrupting Pest Communication

The RNAi-based FPPs major purpose is to lower the population of the forest pest below the level of the epidemic. For insect communication, pheromone is a species-specific chemical molecule. RNAi can be used by silencing the genes involved with the production of sex pheromones to impair the reproductive conduct of the plague. For example, when RNAi silenced two pheromone-binding proteins that reduced the pairing behaviour, *Helicoverpa armigera* could not find female moths. Similarly, genes involved in bark beetle pheromone synthesis can be targeted by RNAi to interfere with communication, such as a pheromone aggregation signal in the *Ips typographus* mass assault. The delivery of dsRNA through trunk injection and/or soil drench molecules may move through the phloem, and the pheromone-binding proteins will be silenced in the beetle upon phloem-feeding.

Enhancing the Stability of dsRNA for Environmental Application

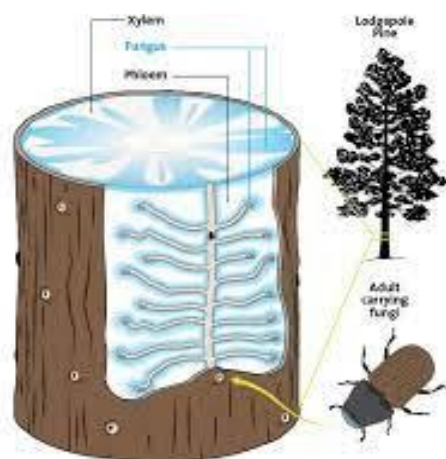
Careful optimisation of goal selection, the design, manufacturing and delivery of dsRNA, nano carriers and symbiotic microorganisms, and the gene silencing caused by viruses may be extremely successful in suppressing and causing deaths in plant RNAi. However, these efforts are not subject to field testing, environment safety and non-target impacts



and the production of diverse RNAi-based FPPs need improvement. However, RNAi based FPPs can support a multiple-species management approach in conjunction with current techniques (i.e. forest management and biological) to preserve the beneficial species of the endemic tree killing populations of forest pests populations in the endemic stage while conserving the beneficial species

Prospects and Challenges for RNAi against Forest Pests

Wood-boring insects thrive inside the bark, trunk on a nutritionally limiting diet.





They tunnel in the inner layer where water and nutrients are available. They attack either healthy or weakened and dead trees based on their statuses like primary invader or secondary invader. It is worth mentioning that primary invaders (i.e., *Ips typographus*, Eurasian spruce bark beetle) mostly kill the infested tree. However, most often, the damage caused by an infestation of wood borers remains unnoticed until the tree showed visible symptoms or external signs of damage, such as the entry hole of a wood borer or sawdust. This hidden lifestyle of wood borers, contrary to most other agricultural pests, causes considerable impediments for control measures, even for RNAi-based FPPs. Perhaps choosing a suitable strategy to deliver dsRNA is a big challenge in RNAi-based forest protection methods. Several possible dsRNA application strategies are available that can be deployed against forest pests. They are transiently transformative (recombinant symbiont or virus) and non-transformative (nanoparticles, trunk injections and spraying, root soaking, and soil drench) methods. Due to the transient feature of the molecules used in non-transformative delivery methods, target pests have limited exposure to the dsRNA molecules, delaying the resistance development. Deploying the RNAi based Plant-incorporated protectants (RNAi-PIPs) via transgenic trees (transformative approach) seems to be a less viable solution against wood-boring forest pests due to public and scientific concern (i.e., gene flow), lack of suitable tree transformation protocols, high development time and cost, and extensive regulatory processes. However, it is worth mentioning here that researchers already developed efficient and stable plastid transformation protocols for poplar, which can be considered for developing RNAi-PIPs against pests infesting the green tissues of poplar.

