

# Characterizing beta-tubulin dsRNA for RNAi control of an aggressive *Neopestalotiopsis* species

Sarah Koeppe<sup>1</sup>, Melanie Kalischuk<sup>1</sup>

<sup>1</sup>Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada N1G 2W1



## INTRODUCTION

*Neopestalotiopsis* sp. is a newly emerging aggressive fungal pathogen of strawberry (*Fragaria x ananassa* (Duch.)) that has been detected in Ontario since 2020 (1). Currently, there are no resistant strawberry cultivars or control methods for the pathogen (1).

RNA interference (RNAi) is a cross-kingdom conserved eukaryotic mechanism triggered by double-stranded RNA (dsRNA) that is currently being explored as a crop protection strategy (2).

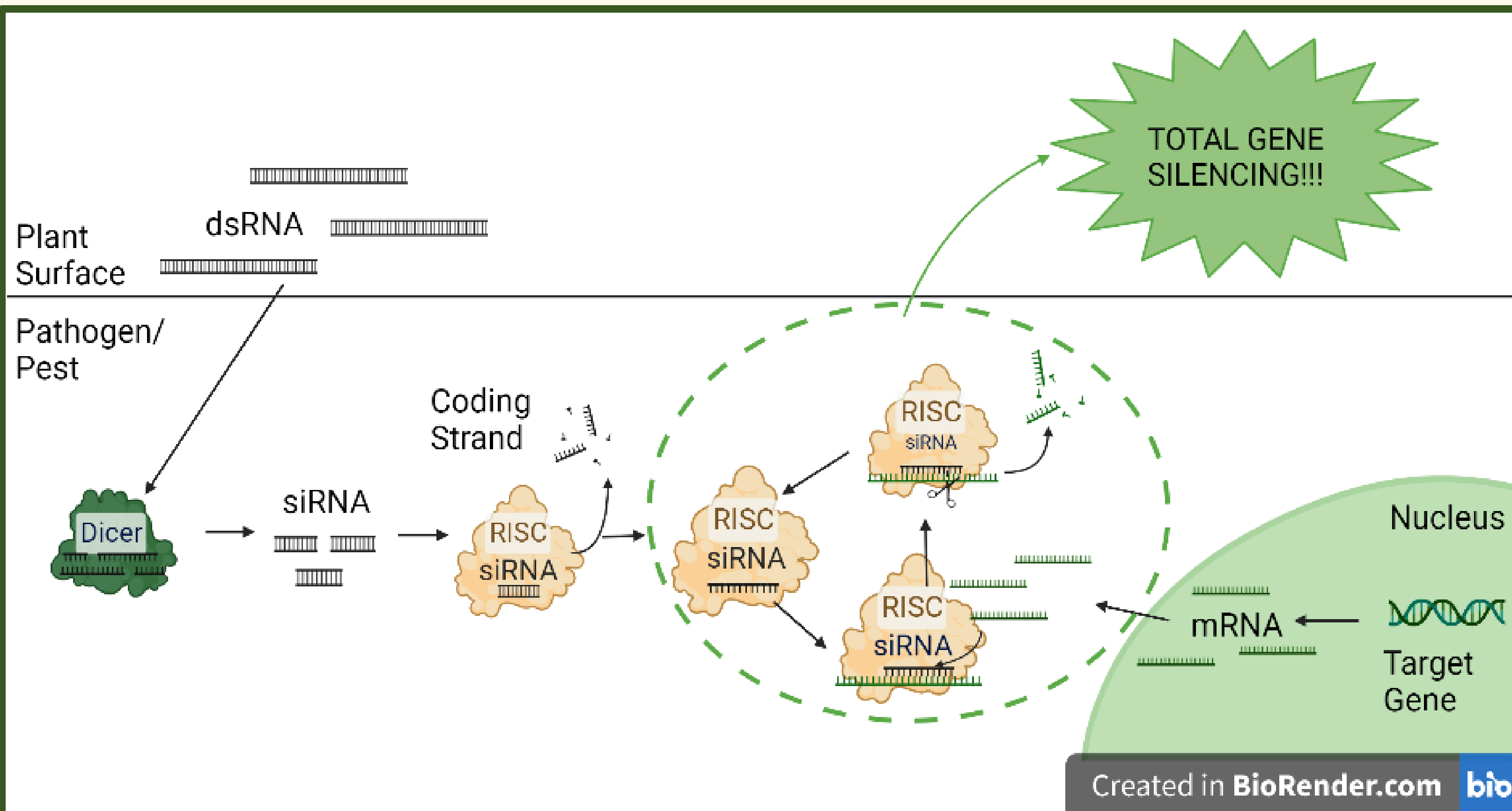


FIGURE 1. Pictured above is a diagram of the RNAi pathway.

Beta-tubulin is an excellent candidate for RNAi as it is a housekeeping gene responsible for producing beta-tubulins, which combine with other tubulins to form tubulin fibres that separate chromosomes during cell-splitting and support cellular structure (3). Beta-tubulin proves to be a good target for fungi specifically as it is the target of site-specific fungicides like benzimidazoles (3). Characterization of this gene is important to designing dsRNA constructs. General rules for designing dsRNA constructs:

1. Avoid regions close to the initiation codon (75-100 bases)
2. Avoid sites that result in multiple secondary structures
3. Avoid introns and 5'UTR or 3'UTR
4. GC content within 30-50%
5. Avoid repeat sections of A's or T's
6. Potentially avoid GGGG or CCCC

## MATERIALS AND METHODS

### Sequence Sources

- Entry PFICI\_14162 (*Pestalotiopsis fici*, tubulin beta chain, a genomic sequence) was utilized as a comparison model as it is well-characterized and highly related to the genus *Neopestalotiopsis*
- *N. rosae* is most closely related to the novel aggressive strain, and therefore GenBank entry JALGAS010000006.1 (contig 6 of a whole-genome shotgun sequencing project, an mRNA sequence) was used as a representative of that strain to characterize the gene

### Finding Introns, Exons, and Splicing Sites

- PFICI\_14162 was compared to JALGAS010000006.1 to pull beta-tubulin from the contig (Nrosae\_Btub)
- The browser EnsemblFungi was utilized as it presents the characterization of genes from databases like NCBI GenBank
- Characterization of entry PFICI\_14162 was aligned with Nrosae\_Btub

### Motif Search

- PFICI\_14162 and Nrosae\_Btub were translated using <https://web.expasy.org/translate/> and run through the program <https://prosite.expasy.org/scanprosite/> to identify motifs

## RESULTS

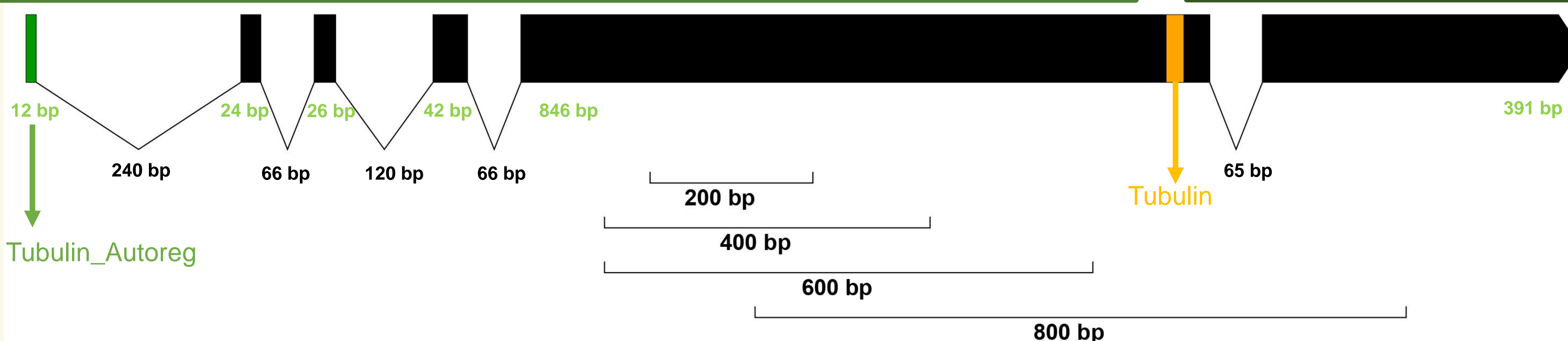


FIGURE 2. Pictured to the left is a diagram of the characterization of Nrosae\_Btub (1910 bp). Solid black bars are the exons (bp count in green), lines connecting are introns (bp count in black), green is the tubulin\_autoreg motif (12 bp), and yellow is the tubulin motif (18 bp). Proposed dsRNA constructs are shown with markers below.

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ATGCGTGAGATTGTGAGTCCATGACTACCCCGACCTTCCCTCTATTACTATACCAACCGACCTACCCTGGACGGCTC
CCAAACTCAATGGCCCTGATTTCTACCCACATCCTTCCGAAAGCTGTCCGCCAAGAGCGGCGGACGAGCAACAG
CGACAACAGATTCCGAAGTCGCTTCATGTCATGACTGCCGTGTAATCAAGTAAATCAAGTTCTCTAGCTA
ACGGGTCTTTTTTCTGCAATAGGTTTACCTTCAGACCGGTCAGTGCCTAAGTAAATGCAAAATCCCGGATATAG
CGGTTCGAAACACAAAGCTCACAATCATGAACAGGTAACCAATTTGGTGCCTTCTGATGTAACCTGTCTGT
CTCGACACGGCCTCAATACGACGTTTTTCGTCGTCACGACGGCCCGAACAGTGAATAGGTCAAGATAGAGGGAA
CATGATGCTAATAGGTCATTGATAGCAAAACCATCTCTGGCGAGCAGGCTTCGACAGCAATGGAGTGTATGTAATTT
TTAATTCCTCTGCTTCCGTGTAAGCTTGTAGGCTGACTCGATGGCCATTAGCTACACGGTACCTCCGAGCTCCAGCT
CGAGCGTATGAGCGTCTACTTCAACGAGGCTTCCGGCAACAAGTACGTTCTCGTGCCTCGTGCATCTCGAGCC
CGGTACCATGGATGCCGTCCGCGCCGGTCCCTTCGGCCAGCTCTTCCGCCCTGACAACCTTCGCTTCGGTCACTCCG
GTGCTGGCAACAACCTGGGCCAAGGTCACACTACACTGAGGGTGTGAGCTCGTCCAGGTCCTCGACGTTGTCCGT
CGCGAGGCGGAGGCTTCCGACTGCCTCCAGGTTTCCAGATCACCCACTCCCTGGTGGTGGTACCGGTGCCGGTAT
GGGTACTCTGTTGATCTCCAAGATCCGCGAGGAGTCCCGACCCGATGATGGCTACCTTCCGCTCGTGCCTCCCG
CAAGGTCTCTGACACCGTCTGACGACCTACAACGCCACCCTCTCCGTCCACCAGCTGGTCGAGAATCCGACGAGA
CCTACTGCATTGACAAACGAGGCTCTTACGACATCTGCATCGTACCCTGAAGCTGCCAACCCCTCTACGGTGAACCT
GAACCACCTGGTCTCCGCGTCATGCTGGCGTCAACCACTTGTCTGGCTTCCCTGGTCAGCTCAACTCTGACCTGGC
CAAGTTGGCTGTCAACATGGTGCCTTCCCGCTGCACTTCTTCATGGTGGCTTGTCTCCGCTGACCAGCCGTGG
CGCTCACTCTTCCGTCGCCGCACTGTTCCGAGTTGACTCAGCAGATGTTCCAGCCCAAGAACATGATGGCTGCTTC
CGACTTCCGCAACCGTCCGTACCTGACTGCTGCTGCACTTGTAAAGTATCTTCCAATTGTTCTAATCGAATCGTATC
ATTCTTGAGCTAACGGGTTTTCTCTAGCGGTGTAAGGTTCTCCATGAAGGAGTTCGAGGACCATGCGCAACGTTCCA
GAACAAGACTCCTCCTACTTGTGAGTGGATCCCAACAACGTCAGACCCGCTCTCTGCTCCATTCTCCCGCGG
CCTTAAGATCTGCTACCTCTGCGGAACTCGACCGTATCCAGGAGCTTCAAGCGTATCCGCGAGCAGTTCACT
GCCATGTTCCGTCGCAAGGCTTCTTGCATTGGTACACTGGTGGGATGGACGAGATGGAGTTCACTGAGGCTGAG
TCCAATATGAACGACTTGGTCAGCGAATACCAGCAGTACCAGGATGCCGGTGTGATGAGGAGGAGGAGTACGAG
GAGGAGCCTCTGCCGAGGACGAGTAA
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FIGURE 3. Pictured above is a diagram containing the Nrosae\_Btub sequence characterized. Red lettering is the start and stop codons, green lettering is the Tubulin\_Autoreg motif, yellow lettering is the tubulin motif, yellow highlight is splicing sites, grey highlight is introns, and green highlight is exons.

200 bp construct  
CAACTTCGCTTCGGTCAGTCCGGTGTGGCAACAACCTGGGCCAAGGTCACACTACACTGAGGGTGTGAGCTCGTCCG  
CCAGGTCCTCGAGTGTCCGTCGCGAGGCGGAGGCTTCCGACTGCTCCAGGTTTCCAGATCACCCACTCCCTGG  
GTGGTGGTACCGGTGCCGGTATGGGTAAGTCTGTTGATCTCCAAG

400 bp construct  
CCCGGTACCATGGATGCCGTCCGCGCCGGTCCCTTCGGCCAGCTTCCGCCCTGACAACCTTCGCTTCGGTCACTCC  
GGTGTGGCAACAACCTGGGCCAAGGTCACACTACACTGAGGGTGTGAGCTCGTCCAGGTCCTCGACGTTGTCCG  
TCGCGAGGCGGAGGCTTCCGACTGCTCCAGGTTTCCAGATCACCCACTCCCTGGTGGTGGTACCGGTGCCGGT  
TGGTACTCTGTTGATCTCCAAGATCCGCGAGGAGTCCCGACCCGATGATGGCTACCTTCCGCTCGTGCCTCCCG  
CAAGGTCTCTGACACCGTCTGACGACCTACAACGCCACCCTCTCCGTCCACCAGCTGGTCGAGAATCCGACGAGAG  
CTACTGCATTG

600 bp construct  
CCCGGTACCATGGATGCCGTCCGCGCCGGTCCCTTCGGCCAGCTTCCGCCCTGACAACCTTCGCTTCGGTCACTCC  
GGTGTGGCAACAACCTGGGCCAAGGTCACACTACACTGAGGGTGTGAGCTCGTCCAGGTCCTCGACGTTGTCCG  
TCGCGAGGCGGAGGCTTCCGACTGCTCCAGGTTTCCAGATCACCCACTCCCTGGTGGTGGTACCGGTGCCGGT  
TGGTACTCTGTTGATCTCCAAGATCCGCGAGGAGTCCCGACCCGATGATGGCTACCTTCCGCTCGTGCCTCCCG  
CAAGGTCTCTGACACCGTCTGACGACCTACAACGCCACCCTCTCCGTCCACCAGCTGGTCGAGAATCCGACGAGAG  
CTACTGCATTGACAACGAGGCTCTTACGACATCTGCATCGTACCCTGAAGCTGTCCAACCCCTTACGGTGAACCT  
AACCCACTGGTCTCCGCGTCACTGCTGGCGTCAACCACTTGTGGTTCCTGGTTCAGCTCAACTCTGACCTGCGG  
AAGTTGGCTGTCAACATGGTGCCTTCCCGCTGCACTTCTTCATGGTCCGG

800 bp construct  
GGGTTCCAGATCACCCACTCCCTGGTGGTGGTACCGGTGCCGGTATGGGTAAGTCTGTTGATCTCCAAGATCCGCGA  
GGAGTTCGCCGACCGCATGATGGCTAAGTCTCCGCTCGTGCCTCCCGCAAGGTCCTGACACCGTCCGTCGAGCCCTA  
CAACGCCACCCTCTCCGTCCACCAGCTGGTCGAGAATCCGACGAGACCTACTGCATTGACAACGAGGCTCTTACGA  
CATCTGCATGGTACCCCTGAAGCTGTCCAACCCCTTACGGTGAACCTGAACCACTGCTCCGCGGTCATGCTGGC  
GTCACCCTTGTTCGCTTCCCTGGTTCAGCTCAACTGACCTGCGCAAGTGGCTGTCAACATGGTGCCTTCCCG  
GTCTGCACTTCTCATGGTCCGCTTGTCCGCTGACCCAGCGTGGCGCTCACTTTCGGTCCGCTCACTGTTCCCG  
AGTTGACTCAGCAGATGTCGACCCCAAGAACATGATGGTGTCTCCGACTCCGCAACGGTCCGTACCTGACCTGCT  
TGCCATCTGTAAGTATCTTCCAATGTTTCTAATCGAATCGATCATTCTTGAGCTAACCGGTTTTCTCTAGCGTG  
AGGTTCCATGAAGGAGTTCGAGGACCATGCGCAACCGTCCGAAACAAGAACTCCTCTACTTGTGTTGAGTGGATCC  
CCAACAAGTGCAGACCGCTCTCTGCTCCATTCCTCCCGCGGCTTAAGATGTCGCTTACTTTCGTCGAAAACCTCGAC  
CGCTATCCAGGA

FIGURE 4. Above are proposed dsRNA constructs for *Neopestalotiopsis* sp.

## CONCLUSIONS AND FUTURE WORK

- Locations of all key elements that characterize beta-tubulin were identified
- dsRNA constructs of various lengths were designed based on the characterization of beta-tubulin
- Future work is to test these dsRNA constructs against *Neopestalotiopsis* sp. to assess efficiency of control

## ACKNOWLEDGEMENTS

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