

## INDICE

<b>Introducción</b>	<b>1</b>
<b>1. Aspectos Generales</b>	<b>3</b>
2.1 Estructura primaria	4
2.2 Estructura secundaria y dominios estructurales	5
<b>3. Localización subcelular</b>	<b>7</b>
<b>4. Replicación</b>	<b>8</b>
<b>5. Movimiento y distribución en la planta</b>	<b>10</b>
<b>6. Clasificación Taxonómica</b>	<b>11</b>
<b>7. Variabilidad de los viroides</b>	<b>15</b>
<b>8. Enfermedades producidas por viroides</b>	<b>17</b>
8.1 La enfermedad del tubérculo fusiforme de la patata	18
8.2 La enfermedad del manchado solar del aguacate	19
8.3 La enfermedad del Cadang-Cadang del cocotero	20
8.4 La enfermedad del enanismo del crisantemo	21
<b>9. Viroides de los Cítricos</b>	<b>22</b>
9.1 El viroide de la exocortis de los cítricos(CEVd)	26
9.2 El viroide del enanismo del lúpulo( HSVd)	28
9.3 El viroide de la hoja curvada de los cítricos( CBLVd)	29
9.4 El viroide del enanismo de los cítricos(CDVd)	30
9.5 El viroide de la corteza agrietada de los cítricos( CBCVd)	31
9.6 El viroide V de los cítricos	31
9.7 El viroide CVd-OS	32
<b>10. Enfermedades de los cítricos producidas por viroides</b>	<b>32</b>
10.1 La exocortis de los cítricos	32
10.2 La caquexia de los cítricos	34
10.3 La enfermedad de la lima Tahití	35
<b>11. Control de las enfermedades producidas por viroides</b>	<b>36</b>
<b>12. Métodos de Detección</b>	<b>36</b>
<b>12.1 Métodos biológicos</b>	<b>37</b>
<b>12.2 Métodos moleculares</b>	<b>39</b>
21.3 Combinación de métodos biológicos y moleculares	43
<b>REFERENCIAS</b>	<b>44</b>
<b>Objetivos</b>	<b>65</b>
<b>Capítulo I</b>	<b>69</b>
<b>A novel Hybridization approach for detection of citrus viroids</b>	<b>71</b>
<b>Abstract</b>	<b>73</b>
<b>Introduction</b>	<b>73</b>
<b>Materials and methods</b>	<b>74</b>
Plant materials and viroid sources	74
Extraction methods	75
RNA analysis by PAGE and sPAGE	75
Northern blot hybridization	75
Probe quantification by Real time PCR	76
<b>Result and discussion</b>	<b>77</b>
Detection of CEVd in field grown sweet orange trees	77
Viroid probe binding properties	78
Specific detection of citrus viroids in field grown sweet orange trees, and other citrus species and cultivars	81
Hybridisation protocol for routine analysis	83
<b>Conclusion</b>	<b>86</b>
<b>References</b>	<b>86</b>

<b>Capítulo II</b>	<b>91</b>
<b>Citrus viroids in Colombia</b>	<b>93</b>
<b>Abstract</b>	<b>95</b>
<b>Introduction</b>	<b>95</b>
<b>Materials and methods</b>	<b>96</b>
Plant materials and nucleic acid extraction	96
Northern blot hybridization	96
RT-PCR analysis	96
Sequencing and sequence analysis	97
Infectivity and bioassay of mutants	97
<b>Results</b>	<b>98</b>
Characterization of CEVd isolates	98
Characterization of HSVd isolates	100
Characterization of CVDD isolates	101
Mutagenesis approach to identify nucleotide position involved in CEVd pathogenesis	103
<b>Discussion</b>	<b>103</b>
<b>References</b>	<b>104</b>
<b>Capítulo III</b>	<b>107</b>
<b>Viroids in Tahiti lime scions showing bark cracking symptoms</b>	<b>109</b>
<b>Abstract</b>	<b>111</b>
<b>Introduction</b>	<b>111</b>
Materials and methods	112
<b>Results</b>	<b>114</b>
<b>Discussion</b>	<b>117</b>
<b>References</b>	<b>119</b>
<b>Capítulo IV</b>	<b>123</b>
<b>Molecular and Biological characterization of natural variants of <i>Citrus dwarfing viroid</i></b>	<b>125</b>
<b>Abstract</b>	<b>127</b>
<b>Introduction</b>	<b>127</b>
<b>Materials and methods</b>	<b>129</b>
Viroid sources	129
cDNA synthesis, PCR amplification and cloning	130
Single-strand conformation polymorphism (SSCP) analysis	130
Sequencing and sequence analysis	131
Infectivity assays	131
Northern hybridization	132
Symptom evaluation and statistical analysis	132
<b>Results</b>	
Symptom expression and plant growth	142
<b>Discussion</b>	<b>144</b>
<b>Acknowledgments</b>	<b>146</b>
<b>References</b>	<b>147</b>
<b>Capítulo V</b>	
<b>Citrus viroids: Symptom expression and performance of Washington navel sweet orange trees grafted on Carrizo citrange</b>	<b>153</b>
<b>Abstract</b>	<b>155</b>

---

<b>Introduction</b>	<b>155</b>
<b>Materials and methods</b>	<b>156</b>
Viroid Sources	156
Plant materials and inoculation	157
Viroid Indexing	159
Symptom evaluation	159
Tree growth and fruit yield	159
Fruit quality	159
Statistical analysis	160
<b>Results</b>	<b>160</b>
Symptoms induced by viroid infection	160
Effect of viroid infection on tree size and fruit harvest	162
Fruit harvest	<b>165</b>
Fruit Characteristics	<b>166</b>
<b>References</b>	<b>180</b>
<b>Anejos</b>	<b>189</b>