大丽轮枝菌对黄栌植株的侵染特点及其定量检测研究

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摘 要：黄栌是著名观赏树种，构成香山红叶的主体景观，对首都的生态经济建设有举足轻重的意义。然而由大丽轮枝菌引起的黄栌枯萎病近年来爆发成灾，严重影响黄栌林健康，成为威胁我国景观建设的重大生物灾害。本文以黄栌盆栽苗为试验材料，通过接种试验、分离培养等基础方法探讨病原菌侵染循环过程，并在黄栌与病原菌互作过程中，测定活性氧含量及相关酶系活性变化，深入研究活性氧在黄栌寄主抗病过程中的作用。此外，借助实时荧光定量 PCR 技术，使用 SYBR Green 染料法，针对大丽轮枝菌 ITS 及 β-tubulin 基因片段，设计特异性引物，建立黄栌枯萎病的实时定量检测体系，并借助该定量体系，探究黄栌枯萎病不同症状与病原菌数量的关系。主要结果如下：

（1）接种方式与病原菌扩展速度及病害严重程度密切相关。蘸根接种寄主发病速度最快，且死亡率最高。灌根接种次之，但发病进程略慢。枝干底端刮伤涂抹可以引起部分植株发生枯萎病症状，但枝干顶端涂抹与叶片接种不能引起病害的发生。病原菌在寄主体内上行侵染。

（2）活性氧水平与黄栌-大丽轮枝菌互作过程密切相关。接种病原菌后，寄主活性氧产生速度增加，且在病害后期有明显积累。活性氧清除酶系活性先升后降，在接种后 15 天达到峰值，与病原菌在寄主体内扩展进程相一致。病害后期活性氧的过量积累，对寄主细胞造成损伤。

（3）建立了大丽轮枝菌的定时定量检测体系。在根据大丽轮枝菌 ITS 区域及 β-tubulin 片段设计合成 4 对特异性引物中，以 ITS 为目的片段的引物 VdF1/VdP1 具有高特异性，可以用于大丽轮枝菌的定量检测。该定量检测体系扩增效率高达 97.05%，具有良好灵敏度，检测下限为 2pg/μl^{-1}。对发病植株新萌生的未显示症状的组织及土壤样品均可检测到病原菌的存在，可有效实现对病害的提前预警。

（4）病害症状与病原菌数量并不存在简单对应关系。以建立的定量检测体系，确定不同症状寄主组织内病原菌数量，结果表明，黄栌枯萎病叶片常见的黄萎及绿枯两类症状中，

病原菌数量并无明显差异。而相较于未显示症状的植物组织，显示症状的植物组织内病原菌数量明显较多，表明症状的显现可能与病原菌数量相关。

关键词：黄栌枯萎病；大丽轮枝菌；活性氧；荧光定量 PCR

Infection Characteristics of Verticillium dahliae on Smoke-tree and its Quantitative Detection Research

Abstract：*Cotinus coggygria* is a famous ornamental tree, which constitute the main scenery of Beijing red leaves during autumn. However, *Verticillium* wilt has emerged in recent years, which seriously affected the forest landscape and health, threatened the ecological environment construction in regions of *Cotinus* production worldwide. It has now considered the most devasting disease in smoke-trees. Since the disease is difficult to control, based on the predecessors research, we systematically exploration about the quantitative detection of pathogen and the pathogen infection charactistics. The main results are as follows:

(1) Inoculating methods and the disease severity are closely connected. Dipping the root in conidial suspension lead the hosts quickly showed symptoms and finally cause the highest mortality. Soil inoculation can also cause the wilt disease, though the incidence process somewhat slower than the root dipping inoculation. Inoculate on the leaves can not lead to wilt symptoms. The pathogen can be found in plant residues during winter.

(2) Test with the spectrophotometers, use 3, 3-diaminobenzidine (DAB), nitro-blue tetrazolium (NBT) and optical microscope to study the effects of infection causing by *Verticillium dahliae* on the oxidative state in the smoke trees. For a period of one month after the inoculation, activities of SOD, POD in smoke tree leaves were changed, firstly increased and then decreased, the value reached maximum at 15 dpi, when the leaves began to show susceptible symptoms. At the same time, production rate of $O_2^-$and H_2O_2 showed increasing trend tendency and obvious accumulation by the late stage of the disease. Observation of cellular localization revealed that accompanied with a huge amount of ROS production in infected Cotinus leaves by late stage of the infection, then the cell died. According to the active changes of SOD and POD, and the main component of ROS content accumulation, it suggested that during early stage of the disease, ROS

may involve in pathogen defense process, whereas when late stage of the disease, ROS may be responsible for cell death.

(3)Establish an effective and rapid quantitative system by the use of specific primer and the SYBR Green dye with real-time PCR technology. The primer targeting the ITS region which can generate an 187bp amplicon, the melt curve show a single dissociation peak at the same temperature proved its specificity. The minimal amount of pathogen DNA quantified is 2pg. It's sensitive enough to detect the pathogen both in the plant issues and the soil, even in the infected tree with symptomless. The results demonstrated that this new quantification PCR assay is reliable, reproducible and sensitive, it's able to accurate detect and quantify the pathogen, predict the risk of disease before a damage threshold and gives prospect to understanding the pathogen spatiotemporal distribution of in the host, facilitate of the manage strategies making.

(4)Use the quantitative system to study the relationship between different symptoms and the pathogen amount. The results showed that there is no straightforward relationship between different disease symptoms and pathogen quantity. But the pathogen in the plant tissues which show symptom is obvious higher than those in the planta look healthy, that indicated the pathogen should reach a certain number before they causes symptom.

Key words: Wilt disease, *Verticillium dahliae*, Reactive Oxygen Species, Real-time PCR