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**PREFORMED AND INDUCED CHEMICAL RESISTANCE OF TEA
LEAF AGAINST *EXOBASIDIUM VEXANS* INFECTION**

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PREFORMED AND INDUCED CHEMICAL RESISTANCE OF TEA LEAF AGAINST *EXOBASIDIUM VEXANS* INFECTION

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ABSTRACT

Levels of (-)-epicatechin in tea cultivars (*Camellia sinensis*) resistant to blister blight leaf disease (*Exobasidium vexans*) were significantly higher than those in susceptible cultivars, while the reverse was true for (-)-epigallocatechin gallate suggesting that epicatechin was involved in the resistance mechanism. The content of the methylxanthines, caffeine and theobromine in the leaf increased significantly in the initial translucent stage of the disease, probably as a defense response to fungal attack. Epicatechin and epigallocatechin levels were significantly less than in healthy tissues at this stage, but increases in the corresponding gallate esters suggested that they were being converted into esters. Although epicatechin and epigallocatechin levels decreased from translucent to mature blister stages, the decrease was not significant. The decrease in levels of epicatechin, epigallocatechin and their esters on infection and the formation of cyanidin and delphinidin on oxidative depolymerization of the blisters suggest that proanthocyanidins may play a role in the defense mechanism. The very high resistance of a purple-green leafed cultivar is attributed to the additional catechin source provided by the high levels of anthocyanins present. Cyanidin and delphinidin were identified as two anthocyanidins in the red tea cultivar TRI 2043.

Infection of leaves of tea which were susceptible to blister blight resulted in a shift of the proanthocyanidin stereochemistry away from 2,3-*trans* (e.g. catechin and galocatechin) and towards 2,3-*cis* (e.g. epicatechin and epigallocatechin). Infection also resulted in increased

gallic acid esterification of the initiating subunits of proanthocyanidins. This was shown by both mass spectroscopy and phloroglucinolysis. Proanthocyanidins isolated from healthy tissue had a predominantly 2,3-*trans* stereochemistry which accounted for 53% and 61% of the total initiating and extension units of proanthocyanidin, respectively. Conversely in infected tissue, proanthocyanidin subunits with a 2,3-*trans* stereochemistry accounted for 26% and 40% of the total initiating and extension units, respectively. Infection had little impact on the hydroxylation state of the B-rings of proanthocyanidins. The products of acid hydrolysis under oxidative conditions had a slight excess of di-hydroxylated B-rings with cyanidin accounting for $58.3 \pm 0.05\%$ and $60.4 \pm 0.2\%$ of the total anthocyanidin recovered following hydrolysis of proanthocyanidin isolated from infected and healthy leaves, respectively. Similar results were obtained by phloroglucinolysis. Thus it is possible that increased resistance of some tea cultivars to may be a result of higher levels of epicatechin or changed proanthocyanidin composition. The occurrence of flavan-3,4-diols (leucoanthocyanidins) in the site of infection was also proved by supplementation experiments with flowers of genetically defined *Matthiola incana*.

Leaves of tea contain extraordinary large amounts of (-)-epigallocatechin, (-)-epicatechin, (+)-gallocatechin, and (+)-catechin and derivatives of these compounds that show positive effects on human health. The health-promoting effects of flavan 3-ols, especially those of green tea, are of scientific and public interest. Furthermore, they play a crucial role in defense against pathogens of tea. Therefore, biosynthesis of these flavonoid compounds was investigated. The anthocyanidin reductase enzyme recently described from *Arabidopsis* and *Medicago* was shown to be present in tea with very high activity and produces epicatechin as well as epigallocatechin from the respective anthocyanidins, thus explaining the very high contents of these compounds. A strong combined dihydroflavonol-4-reductase/leucoanthocyanidin-4-reductase activity was demonstrated and catalyzes the key steps in catechin and gallocatechin formation. Therefore in this study we have elucidated the activities of chalcone synthase (CHS), chalcone isomerase (CHI), flavanone-3- β -hydroxylase (FHT), dihydroflavonol-4-reductase (DFR), leucoanthocyanidin-4-reductase (LAR), flavanone-4-reductase (FNR), and anthocyanidins reductase (ANR) in the tea plant for the first time.