The Influence of Age and Sterol-Inhibiting Fungicides on the Sterol and Steryl Ester Composition of SIF Sensitive and Tolerant Non-Target *Chlorella* Species

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(ABSTRACT)

1-substituted 1,2,4-triazoles form a class of agricultural chemicals known as sterol-inhibiting fungicides (SIFs). These fungicides function through the inhibition of sterol synthesis, which ultimately affects cell membrane fluidity and permeability. Of the two main types of sterols in plants, free sterols (FSs) are thought to be incorporated into membranes while conjugated sterols such as steryl esters (SEs), hypothetically, regulate homeostasis by inserting or removing FSs from cell membranes under changing environmental conditions. Non-target algae species possess sterol synthesis pathways that are affected by a range of SIFs. One of the main objectives of the current study was to determine the reason for the observed sensitivity of *C. fusca* and the tolerance of *C. kessleri* to SIFs relative to total lipid, FS, SE and FFA composition. These parameters were measured using gas chromatography and mass spectroscopy techniques. Both quantitative and qualitative differences in sterol number and type were noted relative to the FS and SE composition of the two species of algae over time. Notably, SEs were detected in both species of algae, although presence and amount varied with the organism. While SEs were more abundant in *C. kessleri*, higher amounts of FSs were found in *C. fusca*. The FS/SE ratios were 64/36 and 88/12 percent of the total sterol in *C. kessleri* and *C. fusca*, respectively. Treatment of *C. fusca* with 2, 4, and 6 ppm and *C. kessleri* with 6,12, and 24 ppm propiconazole caused an accumulation of methylated precursor sterols, resulting in slightly more FSs in both algae. Only 3 of the FSs produced following treatment were different from the control in *C. fusca* while 9 new sterols were found in *C. kessleri*. Treatment also altered the SE fraction in both species, with fewer SEs produced compared to the control, but more novel sterols in *C. kessleri*, suggesting a possible inverse relationship between FSs and SEs in both organisms. Several studies have implicated lipid/sterol concentrations with the potential for cellular bioaccumulation of lipophilic xenobiotics as they relate to membrane permeability. Cell age and environmental parameters can also affect lipid composition of algae. Although cell age did not affect the qualitative sterol composition of *C. fusca* and *C. kessleri*, quantitative differences were observed. Plants exposed to chemical and other environmental stresses accumulate free fatty acids (FFAs), which may be linked to biophysical membrane changes. SIF sensitive *C. fusca*, had inherently higher levels of FFAs than *C. kessleri*. Qualitatively, *C. fusca* exhibited higher percentages of 18:1 and lower ratios of 18:2/18:3 FFAs than *C. kessleri*. In response to increasing SIF treatment, the ratio of 18:2/18:3 FFAs increased in *C. kessleri* and declined in *C. fusca*. The amount of total lipid produced in the cells of *C. fusca* was higher than in *C. kessleri* during all growth stages. Variations were observed in lipid measured as a percent dry weight compared to lipid/cell as the cultures age. Inherent differences in FS, SE, and lipid composition of *C. fusca* and *C. kessleri* as well as age related changes could account for the differences in the susceptibility of the two algae to propiconazole.

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