Title	A Box-Behnken design for predicting the combined effects of relative humidity and temperature on antagonistic yeast population density at the surface of apples
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Abstract

The objective of this work was to develop models predicting the combined effects of relative humidity (RH, 75–98%), temperature (5–25 °C), and initial applied yeast concentration (10^4–10^8 CFU/ml) on the apple-surface population densities of two biocontrol agents fused against postharvest diseases; the antagonistic yeasts *Pichia anomala* strain K and *Candida oleophila* strain O. Experiments were carried out according to a Box-Behnken matrix. Multiple regression analyses showed that both models yielded a good prediction of yeast density. The effect of relative humidity appeared greater than that of temperature. The number of yeast colony-forming units per square centimeter of apple fruit surface increased with increasing relative humidity, temperature, and initial applied yeast concentration. The models predict that under optimal growth conditions (25 °C, 98%), strains O and K should reach a density of 10^4 CFU/cm^2 when applied initially at 2×10^7 (strain O) or 10^7 CFU/ml (strain K). The model results suggest that rainfall was likely the principal cause of the variability of yeast efficacy reported for previous preharvest orchard trials spanning two successive years. Temperature may also contribute to this variation. The models developed here are important tools for predicting population densities of both strains on the apple surface within the experimental limits. The use of these results should contribute to achieving yeast densities of 10^4 CFU/cm^2 on apples by controlling yeast application and environmental factors such as relative humidity and temperature. The results of this study also confirm our previous *in vitro* findings that water activity has a greater effect than temperature on yeast population density.