PRODUCTION OF HUITLACOCHE (*Ustilago maydis*)

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Introduction. Many edible fungi are cultivated throughout the world including: *Agaricus bisporus*, *Lentinula edodes* and *Pleurotus* spp. In central Mexico, kernel galls of the maize fungal pathogen, *Ustilago maydis*, are known as huitlacoche, a highly prized delicacy that has been eaten since Precolumbian times. Commercial production of huitlacoche has received limited study. Age of maize silk and pollination affect the susceptibility of kernels to infection by *U. maydis* (1, 2). Other factors that could affect commercial production of huitlacoche may include: efficient production of inocula, time of inoculation, and ear characteristics of maize hybrids on which huitlacoche is produced. This study reported the influence of these factors for the production of cuitlacoche.

Methods. Sporidial growth was monitored in shake cultures containing individual monosporidial isolates (e.g., a1b1 and a2b2) and a mixture of two compatible isolates in order to determine if inocula could be produced directly from teliospores. Two replicates each of two individual isolates (a1b1 or a2b2) and a mixture of the two isolates (a1b1 + a2b2) were incubated in 500 ml PDB shakes cultures at room temperature. An aliquot of 5 ml was sampled of each experimental unit every 3 h, from 9 to 43 h after seeding PDB with a uniform suspension of each isolate. The concentration of sporidia per ml was counted for each sample with a hemacytometer. The growth of each monosporidial isolate and the mixture of isolates was compared by variance analysis. Two field studies were done at the University of Illinois South Farms. Each study included four replicates of 15 male sterile corn hybrids. Each experimental unit involved four row plot with about 22 plants per 5.1 m row. In the first trial, plants were inoculated 2, 4, 6, 8, 10, and 12 days past mid-silk with a mixture of compatible *U. maydis* isolates (a1b1 and a2b2). About 6 ml of a sporidial suspension was injected down the silk channel with a hand-held spray gun. Severity of infection (% of kernels per ear that formed galls) was measured from all primary ears in a row 19 days after inoculation. In the second trial, plants were inoculated 2 days after mid-silk. Primary ears were harvested 19 days after inoculation from 12 plants in middle two rows of each 4-row plot. Each 24 ear sample was weighed. Ear weight was calculated on a per ear basis. Protection of the ears by husk leaves was rated from 1 to 5 for each ear, where: 1 ≈ ear galls uncovered throughout the ear, and 5 ≈ ear galls completely covered by husk leaves. Husk leaves were removed and quality of galls was rated subjectively from 1 to 5, where: 1 ≈ small galls, unsuitable for huitlacoche and 5 ≈ predominantly large galls, best suited for huitlacoche.

Results and Discussion. Concentration of sporidia increased from 8 x 10^3 per ml after flasks of PDB were first seeded to about 5 x 10^7 per ml after 25 h in shake culture at room temperature. Development of sporidia did not differ among monosporidial isolates (a1b1 or a2b2) and the mixture of compatible isolates (a1b1 + a2b2) when $\log^{10}$ sporidia were analyzed by variance. Concentration of sporidia in shake cultures did not increase substantially between 22 and 40 h. Plants were infected following inoculation in a greenhouse with sporidia produced from the mixed culture (a1b1 + a2b2). Severity of infection (% kernels infected per ear) was affected by time of inoculation, but severity was not different among hybrids. Infection was most severe (about 80%) when plants were inoculated 2 days after mid-silk. Severity of infection decreased about 7.5% for each day that inoculation was delayed. Fewer than 5% of the kernels were infected when plants were inoculated 12 days after mid-silk. Total weight of ears per plot and average ear weight did not differ significantly among hybrids even though average ear weights ranged from 470 and 735 g per ear. Quality of galls and husk protection differed significantly among hybrids. Husk protection was positively correlated with gall quality (r=0.73) but negatively correlated with ear weight (r=-0.75). Similarly, relative maturity of hybrids was positively correlated with husk protection and gall quality but negatively correlated with ear weight. A negative relationship between ear weight and gall quality is perplexing. Ears that weighed more tended to produce many small galls, whereas those with lower weight produced fewer larger galls. Possibly, this relationship was due to the number and size of kernels per ear which may have varied among hybrids. If so hybrids with fewer larger kernels may better suited for production of huitlacoche than those with many small kernels.

Conclusions. The best time of inoculation was 2 days after mild silk appear. Inocula could be produced directly from a mixture of heterogeneous sporidia obtained from germinating teliospores. Characteristics of quality (like gall size and husk protection) and severity of infection depends of the hybrids used to the production of huitlacoche and for the market requirements.

Literature cited

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