OBUYA, JAMES O.[1*], LINDA E. HANSON[2] and GARY D. FRANC[1], [1]University of Wyoming, Plant Sciences Department, Laramie, WY 82071 and [2]USDA-ARS and Michigan State University, East Lansing, MI 48824. **Mating type idiomorphs distribution and their correlation to benzimidazole-resistance in *Cercospora beticola* from the Central High Plains Region, USA**

ABSTRACT

Cercospora leaf spot (CLS) of sugarbeet is caused by *Cercospora beticola* Sacc., and is one of the most destructive foliar diseases worldwide. Fungicides used for disease suppression include benzimidazoles. Resistance to benzimidazoles can develop quickly, and was first reported in *C. beticola* in 1973 from Greece. Despite reduced use, benzimidazole-resistance typically persists in the *C. beticola* population. We investigated the correlation between the distribution of mating types and benzimidazole-resistance in 179 *C. beticola* isolates recovered during prior CLS surveys. Representative isolates (173) were selected from 56 fields in 19 counties and four states in the Central High Plains (2004-2009), and six isolates were from Michigan (2008). Characterization revealed that 104 isolates were sensitive and 69 were resistant to benzimidazole from the region. These isolates were then tested to determine the frequency of mating type idiomorphs by PCR amplification of mating type loci. Results revealed that c.a. 80% of *C. beticola* isolates from the Central High Plains contained the *MAT1-1* idiomorph in contrast to an equal distribution of mating type idiomorphs reported by a research group in Europe. Our results departed from the expected 1:1 ratio except for states that had low sample numbers, such as Wyoming (χ^2 = 1.47, df = 1) and Michigan (χ^2 = 0, df =1, P=0.05), which had equal distributions of mating type idiomorphs. The latter observations are similar to that reported for *C. beticola* in the north central region of the United States. In conclusion, our results showed lack of correlation between the mating type idiomorphs distribution and resistance to benzimidazole in the Central High Plains region. Therefore, sexual recombination may not be occurring in the CHP *C. beticola* population in which benzimidazole resistance is known to persist.

Objective for the research

An investigation of mating type idomorphs and benzimidazole resistance was conducted for the Central High Plains (CHP) *Cercospora beticola* population. This population is characterized by benzimidazole resistance that has persisted despite the fact that application of this fungicide stopped during the late 1990s (Briere *et al.*, 2001 and 2003; personal communication, Western Sugar Cooperative personnel). The mating type idiomorphs distribution was investigated to determine if there was a correlation between mating type idiomorphs and benzimidazole resistance. Results for the mating type idiomorphs distribution can also be used to imply the presence or absence of sexual recombination in the CHP population. Using two unlinked genes, such as mating type and beta-tubulin (target of benzimidazoles) can provide stronger indication about the potential of sexual recombination than the mating type idiomorphs alone (Groenewald *et al.*, 2006).

Materials and Methods

A total of 179 *C. beticola* isolates were analyzed. Representative isolates (173) were selected from 56 fields in 19 counties and four states in the CHP (2004-2009), and six isolates for comparative analysis were from Michigan (2008). Isolates were recovered from sugarbeet leaves following the modified procedure of Briere *et al*. (2001 & 2003). Briefly, air-dried symptomatic leaves collected from sugar beet fields were washed under tap water for 1 hour. At least 12 lesions were then recovered from each leaf under the dissecting microscope. Lesions were sterilized in household bleach (10%) for 1 minute and rinsed with distilled water for 60 seconds. Immediately after rinsing, lesions were transferred onto water agar (2%) plates and incubated under light for 5 days. Single hyphal tips from the growing mycelium were transferred to plates of potato dextrose agar prepared at half concentration and incubated under light for 5-7 days. Mycelia were sub-cultured onto plates containing sugarbeet leaf extract agar (SBLEA) for conidia production.

We used non-amended and amended potato dextrose agar (PDA) plates for the fungicide sensitivity test. Briefly, approximately 14 ml of PDA medium amended with 5 mg L^{-1} of benomyl fungicide was poured into Petri plates. The plates were dried in a laminar flow hood overnight. Amended and non-amended PDA plates were inoculated with three 1 µl evenly distributed drops of conidial suspension. After 7 days each radial growth diameter was measured and an average radial growth diameter was calculated for each isolate. The average radial growth diameter for each isolate on amended PDA was compared that isolate's growth on non-amended PDA. Radial growth diameters in the presence of benzimidazole ≥80% of that isolate's growth in the absence of benzimidazole were insensitive (resistant).

Mycelia harvested from SBLEA were placed into microfuge tubes with c.a. 50 µl of DNA suspension buffer (Tris-EDTA buffer), boiled for 15 minutes to extract DNA, cooled, and centrifuged for 2 minutes to separate mycelia fragments. Finally, the supernatant containing nucleic acid was used for PCR amplification for idiomorphs characterization. Results were analyzed using SAS statistical software version 9.1.3 (SAS Institute Inc., Cary, NC) to determine if there was a correlation between mating type idiomorphs distribution and benzimidazole resistance. Isolates from Michigan were included for comparison.

Results and conclusion

Based on the fungicide sensitivity test, we determined that 104 of the CHP *C. beticola* isolates tested in the study were benzimidazole-sensitive and the remainder 69 was benzimidazole-resistant. We determined that the mating type idiomorphs distribution of the CHP *C. beticola* population deviates significantly from a 1:1 distribution. Also, when we analyzed the benzimidazole-resistant and -sensitive isolates from the same population, we found that each mating type idiomorph distribution deviated significantly from the expected 1:1 ratio. If the assumption that mating type idiomorphs distribution must be within the expected 1:1 ratio to indicate an ongoing sexual recombination is valid, then our results failed to show that sexual recombination is occurring in the CHP region, or that it is infrequent. In conclusion, our results showed lack of correlation between the mating type idiomorphs distribution and resistance to benzimidazole in the CHP region. Therefore, sexual recombination may not be occurring in the CHP *C. beticola* population in which benzimidazole resistance is known to persist.