

Mating type idiomorphs distribution and their correlation to benzimidazole-resistance in *Cercospora beticola* from the Central High Plains Region, USA



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Introduction

Cercospora leaf spot (CLS) caused by *Cercospora beticola* is the most important foliar disease of sugar beet worldwide. An integrated pest management (IPM) strategy is effective for CLS suppression. Fungicides are widely used for disease suppression, and include benzimidazoles. Since benzimidazoles are single mode of action systemic fungicides, resistance can develop quickly in the *C. beticola* population. Benzimidazole-resistance was first reported in *C. beticola* in 1973 from Greece, and later in other regions worldwide. Despite reduced use, benzimidazole resistance typically tends to persist in the *C. beticola* population. Genetic variability and population structure has been reported in *C. beticola* despite the only known reproduction being asexual. Therefore, we investigated the correlation between benzimidazole resistance and mating type idiomorphs distribution in *C. beticola* population.



Fig-1. Adjacent plots (A) with *C. beticola* resistant (left) & susceptible (right) sugarbeet crop and (B) leaves showing developing CLS lesions.

Materials and Methods

- C. beticola* isolates were recovered from sugarbeet lesions and grown on sugarbeet leaf extract agar (SBLEA).
- Benzimidazole sensitivity of each isolate was determined on potato dextrose agar amended with 5 mg L⁻¹ of benomyl fungicide.
- Mycelia were harvested from SBLEA plates and placed into microfuge tubes with c.a. 50 µl of DNA suspension buffer (Tris-EDTA buffer).
- Mycelial suspensions were boiled for 15 minutes to extract DNA, cooled, and centrifuged to separate mycelia fragments from supernatant.
- The supernatant was used for PCR amplification using two pairs of primers designed from mating type idiomorphs sequences (Groenwald *et al.*, 2006).
- Results obtained from PCR amplification were analyzed using SAS to determine mating type idiomorphs distribution.

Results

Table-1. Mating type idiomorphs distribution in the Central High Plains region.

	N ^a	MATI-1	MATI-2	Ratio ^b	χ^2 ^c	P ^d
by Year^e						
2004	22	17(0.77)	5(0.23) ^e	3.4	6.55	0.011
2005	20	17(0.85)	3(0.15)	5.6	9.8	0.002
2006	19	14(0.74)	5(0.26)	2.8	4.26	0.039
2007	8	3(0.38)	5(0.62)	0.6	0.5	0.48
2008	23	19(0.83)	4(0.17)	4.8	9.8	0.002
2009	81	74(0.91)	7(0.09)	10.6	55.42	0
by State						
CO	20	16(0.80)	4(0.20)	4	7.2	0.007
MI ^f	6	3(0.50)	3(0.50)	1	0	1
MT	45	36(0.80)	9(0.20)	4	16.2	0
NE	91	81(0.89)	10(0.11)	8.1	55.4	0
WY	17	11(0.65)	6(0.35)	1.8	1.47	0.225
by Benzimidazole Phenotype^g						
Resistant	69	58 (0.84)	11 (0.16)	4.7	32	0.0001
Sensitive	104	86 (0.83)	18 (0.17)	4.4	44.6	0.0001

a. Number of isolates tested.

b. MATI-1:MATI-2 ratio.

c. Chi-square value for normal distribution of MATI-1:MATI-2.

d. Probability of goodness of fit to the expected 1:1 ratio of MATI-1:MATI-2 (Critical value of $\chi^2_{df=1} = 3.84$ at $P=0.05$).

e. Numbers in brackets represents the frequency of each loci.

f. Michigan was included for comparison.

g. Michigan data was excluded from the analysis.

Fig-2. Percentage of mating type idiomorphs distributed by state in Central High Plains region.

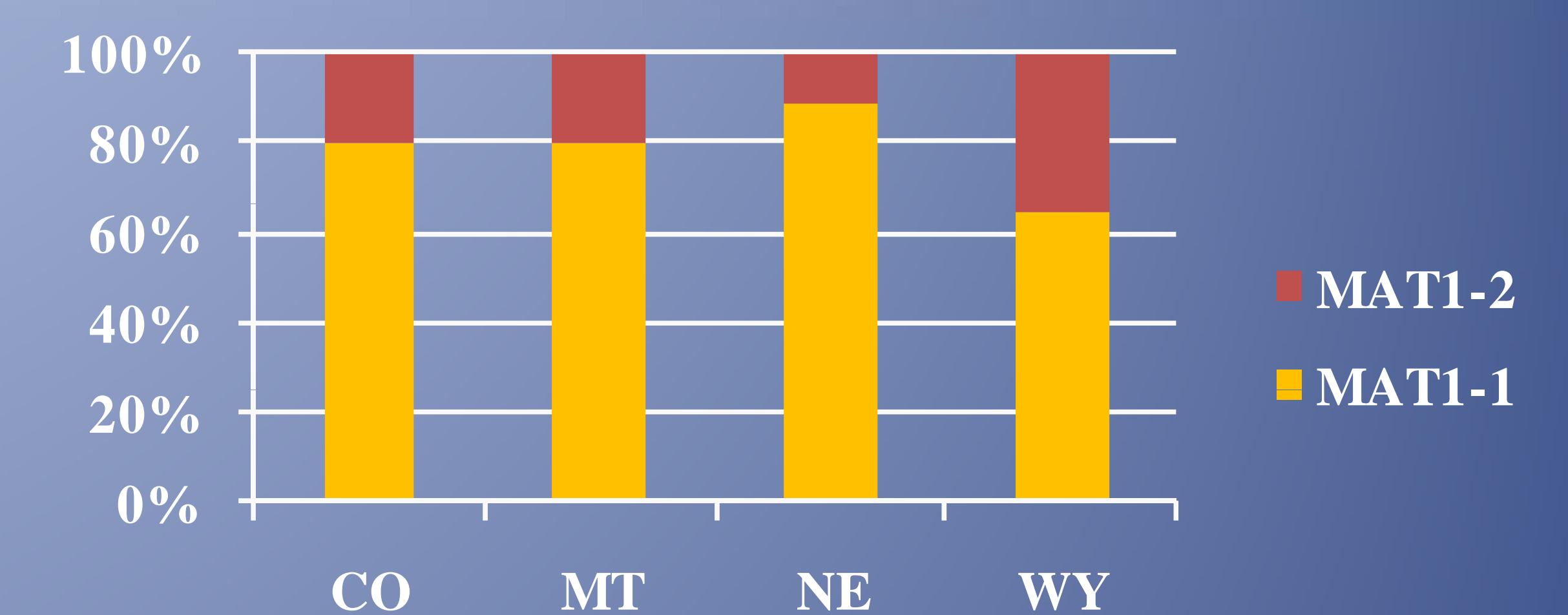
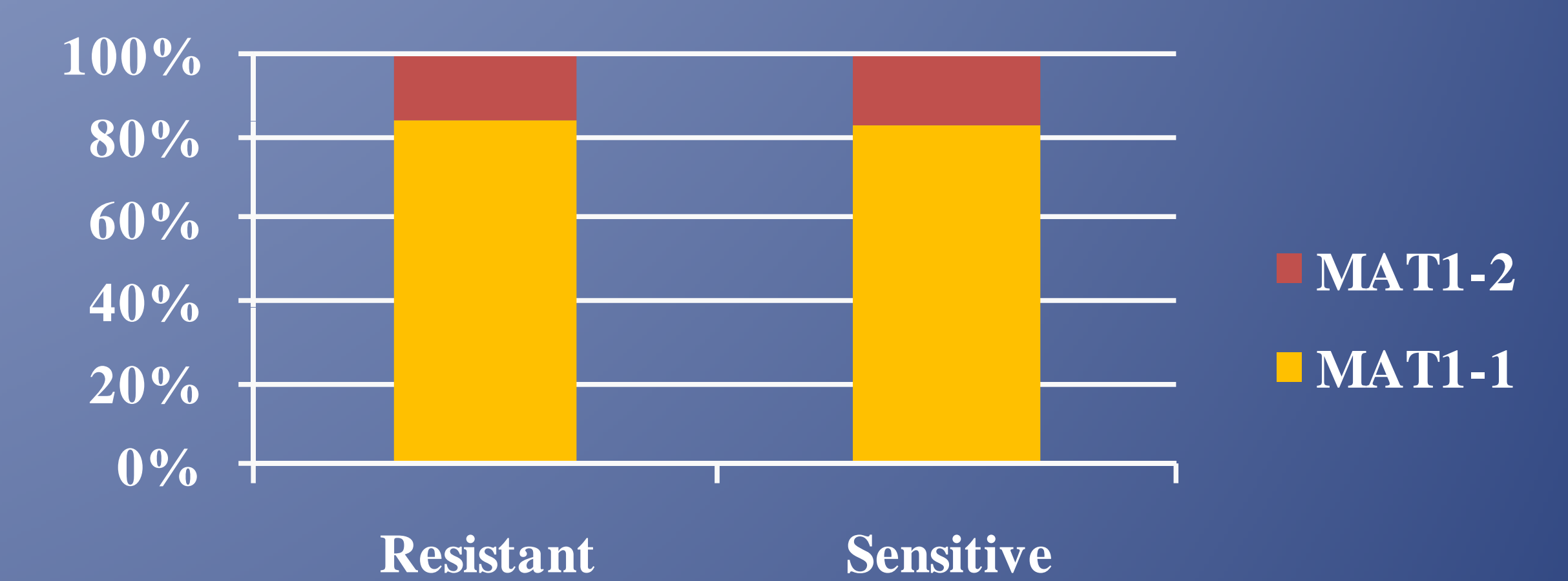


Fig-3. Percentage of mating type idiomorphs distributed by benzimidazole sensitivity in the Central High Plains region.



Conclusion

- The result showed that Central High plains *C. beticola* isolates were approximately 80% MATI-1 as compared to 20% MATI-2, which significantly deviated from the expected 1:1 ratio.
- Also, the result of mating type idiomorphs distribution by benzimidazole sensitivity and resistance significantly deviated from the expected 1:1 ratio.
- If the assumption is valid that sexual recombination leads to a 1:1 distribution of mating type, our results fail to indicate that sexual recombination is occurring in the CHP region, or that it is infrequent.
- Therefore, sexual recombination may not be occurring in the Central High Plains *C. beticola* population in which benzimidazole resistance is known to persist.

References

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