The Effects of Host Diversity in Anthracnose Management and Race Composition of *Colletotrichum sublineolum* in Sorghum Lineage Mixtures

Henrique Maia Valério¹, Janete Maria Silva Alves¹, Maria Aparecida Resende², Elio Gomes Fernandes¹ and Carlos Roberto Casela³

1. Laboratório de Ecologia de Microrganismos e Microbiologia Ambiental, Centro de Ciências Biológicas e da Saúde, Universidade Estadual de Montes Claros (UNIMONTES), Montes Claros 39401-089, MG, Brazil

2. Laboratório de Micologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte 31270-901, MG, Brazil

3. Embrapa Milho e Sorgo, Caixa Postal 151, Sete Lagoas 35701-970, MG, Brazil

Abstract: Alternative strategies of management of sorghum diseases were implemented using field experiments conducted in Embrapa Maize and Sorghum Research Center, Brazil in 2001 until 2003 to evaluate the efficacy of lineage mixtures of *Sorghum bicolor* for the control of anthracnose (caused by *Colletotrichum sublineolum*). The calculated values for disease severity considered the area under the disease progress curve (AUDPC) observed for the nine susceptible and resistant elite strains of sorghum planted in 30 stands randomly. Each stand was drawn in mixtures of three genotypes each, in the same proportions per plant. This design was compared with the severity of the disease measured in AUDPC calculated for each of the same nine strains of sorghum in pure stands. In most of the cases, the disease severity on the susceptible cultivars was decreased; and in some cases, reached 85% of disease reduction compared with disease progress in pure stand of correspondent plant. The implication and effect of these sorghum mixtures on the populations of *C. sublineolum* on each treatment were evaluated and revealed that there was an increase of frequency of complex races in most of these mixtures and a highest phenotypic diversity for virulence to pathogen populations from pure stands, than the characterized on populations collected from the mixtures, despite of the effectiveness on decrease of the anthracnose severity.

Key words: Sorghum mixtures, anthracnose severity, virulence diversity, simple and complex pathotypes.

1. Introduction

Sorghum anthracnose, caused by *Colletotrichum sublineolum* (Ces.) Wils. (Syn. *C. sublineolum* P. Henn) is the most important disease affecting this crop in Brazil. The disease can reduce yield of sorghum substantially and is particularly damaging when susceptible cultivars are grown in areas, where warm and high relative humidity predominate throughout the season. Although the most practical and effective method of controlling the disease used remains the

genetic resistance available with hybrids employed in sorghum growing areas from Brazil, these resistances have been, sometimes, nondurable because of the high variability present in populations of this pathogen [1-3]. Many studies of the effects of host genotype diversity on diseases are in the early exploratory phase for many host-pathogen systems [4], and some works have reported possible alternative improves in the use of the diversification of different *Sorghum* spp. genotypes in mixtures way that was tested for the control of anthracnose and leaf blight [5-7]. Employment of genetic diversification of host for disease resistance is expected to stabilize pathogen

Corresponding author: Henrique Maia Valério, professor, research fields: applied microbiology and microbial ecology.

populations, thereby preventing the erosion (breakdown) of new disease resistance. The strategies of mixtures are being used increasingly in commercial production around the world [8]. Many of the initial studies and applications of mixtures in modern agriculture were multiline cultivars. Despite the fact that multilines are still being used today, there has been a move in favor of cultivar mixtures over multilines. Cultivar mixtures are advantageous because they require no additional breeding effort and allow incorporation of agronomically superior cultivars as they become available [9]. Although there is extensive literature about the use of multiline cultivars and cultivar mixtures to manage disease [10-12], the vast majority concerns pathosystems, in which pathogenicity and resistance vary qualitatively [13]. In particular, the study of pathogen evolution within mixtures has been almost exclusively confined to such pathosystems. Little is known about the selective effects of cultivar mixtures on pathogen evolution in disease system where resistance is partial. Despite the fact that some authors found little benefit from mixing barley cultivars to manage *Rhynchosporium secalis*, a splash-dispersed pathogen [14], like *C. sublineolum*, several papers reported the advantageous to use cultivar mixtures to reduce anthracnose severity in the *Sorghum bicolor*-*C. sublineolum* pathosystem [5-7] and a relative increase in yield production, when compared the mixtures against pure stands crop [8, 15].

Many of the theoretical and empirical investigations of pathogen evolution in mixtures have concerned the potential for selecting complex races (those possessing multiple virulence genes), the relative fitness of complex and simple races and the extent to which complex races, if favored, could erode the benefits of multilines and cultivars mixtures [8, 11, 16-21]. Like other pathosystem formed by *Mycosphaerella sublineolum*-wheat [22], the *C. sublineolum*-sorghum pathosystem is useful for addressing these issues, because resistance in most

cultivars is partial. Additionally, the *C. sublineolum* population on sorghum is highly genetically diverse, and there has been insufficient investigation of the potential to manage sorghum anthracnose with cultivar mixtures and how this approach will affect the composition, i.e., the virulence of the pathogen population, and if the phenotypic diversity (how many hosts each one of the *C. sublineolum* isolates could infect, both in the field and in the greenhouse experiments) is the same in this sorghum lineage as in mixtures and in pure stands.

So, the aim of this work was to determine the effects of the host diversify generated by different three-component combination of sorghum lineages regarding anthracnose severity, the frequencies of each pathotype and pathogen population diversity of the *C. sublineolum* isolates from mixtures in relation to the pure stands of each sorghum genotype.

2. Materials and Methods

2.1 Plant Materials

Sorghum was selected for this study primarily because it does part of the breeding program from Embrapa Maize and Sorghum Research Center in Brazil. The main consideration in selecting these nine sorghum lineages used in this study: CMSXS210A, CMSXS112A, CMSXS215A, CMSXS221A, CMSXS169R, CMSXS180R, CMSXS182R, CMSXS227R and CMSXS116R was basically, the distinct level of resistance to *C. sublineolum*, measured in several fields and greenhouse experiments of sorghum lineages used in the breeding program of Embrapa, in different geographical locations where sorghum were cropped along Brazil. Other considerations in choosing these components in the mixtures were because of their similarity in maturity and yield and their easily identifiable characters, such as grain color (red and white) and plant pigment in response to injury. These differences made it possible to identify the genotype of each plant when taking disease notes.

2.2 Mixtures

Seeds were mixed manually before planting and placed separately in envelopes corresponding to each row for sowing. The number of seeds of each component in the mixtures and the pure stands was adjusted by the percentage of seed germination. Mixtures and pure stands of each component lineage, totaling 39 treatments (Table 1) were arranged in a randomized complete block design with three replications. Mixtures and pure stands were planted in two row plots, separated by two rows of the resistant genotype SC283 to reduce interplot interference, and the individual plot consisted of four rows of 5 m long, a compromise spacing of 70 cm was adopted in all plots with 0.15 m between plants in a standard crop management procedures. No susceptible strains of sorghum were used between the lines both in mixture and in pure plots. The experiments were conducted at the field experimental area of Embrapa Maize and Sorghum Research Center (44°10'30" W, 19°27'18" S approximately, south hemisphere) in the end of March, at the final of summer season, with poor humidity, but weekly irrigation with a water aspersion system.

2.3 Data Collection

All experiments relied on natural inoculum for disease development. On 60 d after cropping, to each assessment date, seven in totality, one per week, five plants of each sorghum lineage were sampled for disease severity in each plot. The severity of anthracnose was estimated by the percentage of area affected on the basal, mean, apical and flag leaves of each plant [6]. For statistical analysis of severity percentage, the corresponding area under the disease progress curve (AUDPC) for the leaves of each plant was calculated using an AUDPC program [23]. The effects of mixtures on disease severity of all treatments were assessed by analysis of variance (ANOVA), and Tukey-Kramer procedure at $p < 0.05$ was carried out for statistical comparison of means based on AUDPC values using GENES program [24].

Table 1 Treatments in the study of the effects of host diversity as a disease management practice composed by *S. bicolor* in pure stands and the correspondent mixtures of three components of each lineage on anthracnose caused by *C. sublineolum*.

Treatments	Sorghum lineages and mixtures
1	CMSXS210
2	CMSXS112
3	CMSXS215
4	CMSXS221
5	CMSXS169
6	CMSXS180
7	CMSXS182
8	CMSXS227
9	CMSXS116
10	210 × 112 × 169
11	210 × 215 × 169
12	210 × 221 × 169
13	112 × 215 × 169
14	112 × 221 × 169
15	215 × 221 × 169
16	210 × 112 × 180
17	210 × 215 × 180
18	210 × 221 × 180
19	112 × 215 × 180
20	112 × 221 × 180
21	215 × 221 × 180
22	210 × 112 × 182
23	210 × 215 × 182
24	210 × 221 × 182
25	112 × 215 × 182
26	112 × 221 × 182
27	215 × 221 × 182
28	210 × 112 × 227
29	210 × 215 × 227
30	210 × 221 × 227
31	112 × 215 × 227
32	112 × 221 × 227
33	215 × 221 × 227
34	210 × 112 × 116
35	210 × 215 × 116
36	210 × 221 × 116
37	112 × 215 × 116
38	112 × 221 × 116
39	215 × 221 × 116

2.4 Isolates

The pathotype diversity and complexity of the populations of *C. sublineolum* were evaluated in each treatment. An attempt was made to obtain the isolates

per leaf, basically from a sporulating midrib lesion. Diseased plant material was stored in labeled envelopes until the isolation of fungal material. From each sample, several small symptomatic leaf pieces were surface-sterilised in 2.0% sodium hypochlorite for 3-5 min before being placed onto oatmeal agar (OMA; 60 g of oat meal and 15 g of agar per litre) under continuous fluorescent light at 25 °C. After 5 d, the growing mycelium was scraped with a platinum ring, further being maintained under continuous fluorescent light at 25 °C for 7 d. When sporulation occurred, these were transferred to tubes, containing sterile distilled water to make a conidial suspension of 10^4 conidia/mL. An aliquot 1 L of this suspension was transferred to Petri dishes containing 2% water-agar. For germination, plates were incubated under continuous fluorescent light at 25 °C for 12 h. Individual germinated spores were outlined on the medium by a wire loop fastened below the low power objective of a dissecting microscope. The outlined spore was examined for the presence of other spores, and if none was observed, the selected spore was transferred to separate OMA culture tubes. After development, single conidial cultures were flooded with sterile mineral oil for maintenance. One conidium per leaf per plant was obtained in each treatment and kept at 25 °C.

2.5 Virulence Diversity

The five of these nine sorghum lineages used as component of the mixtures were chosen as differentials regarding characteristic symptoms of resistance or susceptibility against *C. sublineolum* easily recognizable: CMSXS210A, CMSXS112A, CMSXS221A, CMSXS180R and CMSXS116R. Lineages were planted in the greenhouse in a split-plot arrangement using a randomized complete block design, with isolates randomized as whole plots and sorghum genotypes as sub-plots. Due to the limitation of space in the greenhouse, isolates were inoculated in groups of 10. One experiment consisted of five

differentials and 10 isolates in three replications. One pot containing 5-6 plants was considered as a replication. The races of *C. sublineolum* were characterized by their virulence on this set of sorghum. To increase the yield of conidia for inoculations, each single conidial isolate was transferred to OMA plates and incubated for 7 d under continuous fluorescent light at 25 °C. Plates were flooded with 5 mL sterilized distilled water and the colony surface was scraped with a spatula to dislodge conidia. The conidial suspension was filtered through two layers of cheesecloth and the volume was adjusted to 10^6 conidia/mL. For the adjustment of the spore suspension, 16 fields of 0.1 mm^3 were counted per isolate on an improved Neubauer Hy-Lite Hemacytometer (Hausser Scientific). Three drops of Tween 20 (Merck, Darmstadt, Germany) were added per litre to each suspension as a wetting agent. Inoculums were applied with a hand sprayer on the leaf surface of 30 d old plants, grown in plastic pots in a greenhouse. After inoculation, plants were incubated for 18 h in the dark at 25 °C in a dew deposition chamber at 100% relative humidity.

Disease symptoms were scored 14 d after inoculation, with 1 to 5 scale representing different infection types [25]: (1) presence of chlorotic flecks; (2) red spots on the leaf lamina; (3) necrotic lesions, sometimes elongated, but no acervuli formed; (4) necrotic lesions formed with acervuli present in the centre; (5) necrotic lesions, sometimes coalescing with abundant formation of acervuli.

Infection types were separated into two classes: R = resistant (infection types 1, 2 and 3) and S = susceptible (infection types 4 and 5).

Each isolate was assigned a value based on the binary nomenclature system [2]. Each cultivar had an assigned number ($2n$), where n corresponded to the place occupied by the cultivar within the differential set, which determined the correspondent race group. Designation of a race number was obtained by summing the $2n$ values of all cultivars that exhibited

susceptible reactions to each isolate. Population phenotypic diversity (virulence) was evaluated through the Shannon diversity index [26], among which *C. sublineolum* isolates were obtained from these treatments regards to race frequencies (%) on each *S. bicolor* lineages in mixture in relation to race frequencies characterized for each isolate obtained from each sorghum genotype in pure stand.

3. Results

Virulence phenotypes of *C. sublineolum* were variably distributed throughout treatments. A total of 21 pathotypes were detected among 400 isolates collected from pure stands and three-component mixtures (Table 1). The analysis of the effects of these mixtures based on the anthracnose severity indicated that it was significantly reduced in all susceptible sorghum lineages present in mixtures of three components, as compared to anthracnose severity measured on each sorghum lineage when planted in pure plots. Linear effects were significantly ($p < 0.05$) distinct for the AUDPC values for mean of all observed leaves of each sorghum lineage from mixtures when compared with the disease severity from the pure stand of each sorghum lineage (Table 2). The AUDPC mean values of each mixture, considering the average of three components additionally, show that even the sorghum lineages fewer susceptible to *C. sublineolum* presented a decrease in anthracnose severity in mixtures, as compared to the their respective AUDPC values in pure stand (CMSXS210A and CMSXS215A). As example, for the CMSXS112A in combination with CMSXS215A and CMSXS169R, the AUDPC values reduce in 72% in relation to 112A sorghum lineage in pure stand. Based on the decrease of anthracnose severity observed on the mixtures shown in Table 2, there was a wide variation among these mixtures, and the differences regarding disease severity from several mixtures were significant at $p < 0.05$ level. To the AUDPC values calculated separately for each

component in mixtures, the reduction in disease severity was statistically significant for AUDPC values for most of these sorghum lineages in mixtures in relation to the AUDPC for each component in pure stand (data not showed). The effects of the mixtures on reduction of the anthracnose severity were more pronounced on susceptible sorghum lineages used in this study, independently of the elite male sorghum lineages (e.g., CMSXS169R, CMSXS180R, CMSXS182R, CMSXS227R and CMSXS116R) used in sorghum breeding program of Embrapa Maize and Sorghum Research Center that showed more resistance against anthracnose (Figs. 1-5).

In all sorghum lineage mixtures, a reduction in phenotypic diversity and an increase in frequency of more complex races were observed in relation to the pure stands of each sorghum lineage, especially in treatments involving lineages CMSXS210A, CMSXS215A, CMSXS112A, CMSXS221A and CMSXS180R (Figs. 6-8). The complex pathotypes of *C. sublineolum* isolates increased in frequency, but its rate of increase was reduced when intra-pathotypes showed virulence diversity, a fact observed for each sorghum lineage sowed in pure stand. Therefore, when intra-pathotype diversity was reduced, in isolates collected from the sorghum lineages in these mixtures, the complex pathotype increased faster in frequency. Although these effects were probably due to occurrence of directional selection from the mixtures, this fact had not been coupled within increase in aggressiveness, because in most of these cases, there was a decrease in anthracnose severity from the mixtures, even for more resistant sorghum lineages CMSXS182R, CMSXS227R and CMSXS116R on each of the correspondent mixture (Table 2). The disease incidence in this field experiments measured in susceptible and in more resistant sorghum lineages in each pure stands was always greater than those disease incidence observed on these mixtures with resistant sorghum lineages (data not shown). These results indicated how the

Table 2 ANOVA applied to AUDPC means for anthracnose among nine sorghum lineages in single plot and 30 correspondent mixtures of three components of lineages by Tukey test at 0.05 of probability (significance).

Variable: AUDPC					
Treatments ¹	AUDPC values	$p > 0.05^*$	Treatments ¹	AUDPC values	$p > 0.05^*$
2	802.2	a	1	8.7	g
4	687.2	a	2	802.2	a
6	390.1	b	3	13.4	g
8	337.2	bc	4	687.2	a
20	252.5	bcd	5	2.0	g
9	244.1	bcd	6	390.1	b
32	235.5	bcde	7	230.4	bcde
7	230.4	bcde	8	337.2	bc
38	225.4	bcde	9	244.1	bcde
14	218.3	bcde	10	87.1	defg
26	208.0	cde	11	3.5	g
19	197.8	cdef	12	108.7	defg
24	165.5	cdefg	13	78.9	efg
28	158.2	defg	14	218.3	bcde
16	139.6	defg	15	117.8	defg
34	133.2	defg	16	139.6	defg
31	130.0	defg	17	22.1	g
18	120.9	defg	18	120.9	defg
22	119.9	defg	19	197.8	cdef
15	117.8	defg	20	252.5	bcd
30	114.6	defg	21	106.9	defg
36	114.3	defg	22	119.9	defg
27	113.1	defg	23	19.1	g
33	111.9	defg	24	165.5	cdefg
37	110.2	defg	25	97.5	defg
12	108.7	defg	26	208.0	cde
21	106.9	defg	27	113.1	defg
25	97.5	defg	28	158.2	defg
39	89.9	defg	29	29.4	fg
10	87.1	defg	30	114.6	defg
13	78.9	efg	31	130.0	defg
29	29.4	fg	32	235.5	bcde
35	22.5	g	33	111.9	defg
17	22.1	g	34	133.2	defg
23	19.1	g	35	22.5	g
3	13.4	g	36	114.3	defg
1	8.8	g	37	110.2	defg
11	3.5	g	38	225.4	bcde
5	2.0	g	39	89.9	defg
Mean	163.3	Coefficient of variation (%)		32.0	
Low value	0.8	Higher value		858.8	
DMS-Tukey (1%)	194.2	DMS-Tukey (5%)		172.3	

* Means followed by the same letter are not differentiated by $p > 0.05$ at Tukey-Kramer test.

¹ Means of sorghum lineages in pure plots and in mixtures (treatments).

Q.M.R. (2,733.9) is the estimator of the residual average variance, e.g., Q.M.R. stands for mean square of ANOVA residues; q (5.7) means sample size of the groups; DMS (172.3) means minimal significant difference, that is, to compare all possible pairs of means obtained by treatment.

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Fig. 1 AUDPC of sorghum lineages used in this mixture versus AUDPC in pure stands.
1: lineage 210A, 2: lineage 112A, 3: lineage 180R (x axis).

Fig. 2 AUDPC of sorghum lineages used in this mixture versus AUDPC in pure stands.
1: lineage 210A, 2: lineage 221A, 3: lineage 180R (x axis).

Fig. 3 AUDPC of sorghum lineages used in this mixture versus AUDPC in pure stands.
1: lineage 112A, 2: lineage 215A, 3: lineage 180R (x axis).

Fig. 4 AUDPC of sorghum lineages used in this mixture versus AUDPC in pure stands.

1: lineage 215A, 2: lineage 221A, 3: lineage 180R (x axis).

Fig. 5 AUDPC of sorghum lineages used in this mixture versus AUDPC in pure stands.

1: lineage 112A, 2: lineage 221A, 3: lineage 180R (x axis).

Fig. 6 Complexity of *C. sublineolum* on sorghum lineage (number of susceptible differentials) CMSXS112 in pure stand (white), in mixture with CMSXS210 and CMSXS215 (gray), and in mixture with CMSXS221 and CMSXS180 (black).

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Fig. 7 Complexity of *C. sublineolum* on sorghum lineage (number of susceptible differentials) CMSXS221 in pure stand (white), in mixture with CMSXS210 and CMSXS215 (gray), and in mixture with CMSXS112 and CMSXS180 (black).

Fig. 8 Complexity of *C. sublineolum* on sorghum lineage (number of susceptible differentials) CMSXS180 in pure stand (white), in mixture with CMSXS210 and CMSXS215 (gray), and in mixture with CMSXS112 and CMSXS221 (black).

Fig. 9 Phenotypic diversity measured for the isolates collected from the mixture formed by the components 210A + 112A + 180R and from the sorghum lineages 112A and 180R in pure stand.

Fig. 10 Phenotypic diversity measured for the isolates collected from the mixture formed by the components 210A + 221A + 180R and from the sorghum lineages 221A and 180R in pure stand.

Fig. 11 Phenotypic diversity measured for the isolates collected from the mixture formed by the components 215A + 112A + 180R and from the sorghum lineages 112A and 180R in pure stand.

Fig. 12 Phenotypic diversity measured for the isolates collected from the mixture formed by the components 215A + 221A + 180R and from the sorghum lineages 221A and 180R in pure stand.

Fig. 13 Phenotypic diversity measured for the isolates collected from the mixture formed by the components 112A + 221A + 180R and from the sorghum lineages 112A, 221A and 180R in pure stand.

pathogen population evolved in terms of genotypes and pathotypes on each mixture, indicating that there was a tendency to replace the simple races with complex races, evidencing that the mean multiplication rate in the mixture increased faster for complex pathotypes than for simple pathotypes, when rarely simple races were characterized from the sorghum lineages on each mixture.

The pathotype diversity was always higher for the isolates collected from the pure stands of each sorghum lineage when compared to the Shannon index measured for the pathogen population of *C. sublineolum* isolates collected from each sorghum lineage in these mixtures (Figs. 9-13). Except for the mixture of the components CMSXS112A, CMSXS221A and CMSXS180R, where only the pathotype with virulence for four lines of sorghum was characterized, the Shannon index verified from these sorghum lineages was scored as 0 (zero). The increase in the relative frequency of the race 31 (pathotype with virulence to five elite lines of sorghum) on some of this mixtures and decrease in the frequency of the simple races keep the pathotype diversity low based on the Shannon values for most of the mixtures.

4. Discussion

The effects of these sorghum lineage mixtures can reduce development of sorghum anthracnose in the field, based on the results from these experiments.

Both anthracnose incidence (data not shown) and severity were consistently lower in mixtures of three components than those containing even only one component of resistant sorghum lineage. The AUDPCs of these mixtures with sorghum lineages based on the disease severity notes were always lower than those of the susceptible and resistant cultivars in pure stands, except for the CMSXS169R that showed a higher anthracnose severity in some mixtures, as compared with their respective anthracnose severity measured from the pure stands. The effectiveness of mixtures in restricting epidemic development has been widely reported in small grains infected by rusts, powdery mildews, and even for splash-dispersed pathogens [27], like *C. sublineolum* [6, 7]. In fact, the ephemeral effectiveness of resistance to variable pathogens, such as *C. sublineolum* has demanded considerable efforts in search for a more durable resistance and the diversification of the host population, as a way to stabilize the pathogen population and to slow down the rate of disease development [28, 29]. Despite the fact that the effectiveness of host mixtures, in a more efficiency scale, from randomized mixtures, such as in the present study, for controlling epidemics is well documented in the literature [12], even for sorghum mixtures [5-7, 30], very few works trying to answer the question on the evolution of pathogen populations in response to host mixture are still available [31, 32]. Differently as demonstrated by some authors [33-36],

like shown in results, each one of the component of these mixtures did not support more diverse pathogen populations than those sorghum lineages in pure stands. In the longer term, the question is which mixture will be selected for increasing frequency of complex races, i.e., those with virulence corresponding to more than one resistance gene in the mixture [37], exactly as observed on results of this paper. As was observed by some researchers, done in small-scale experimental plots, the relative frequency of complex *Blumeria graminis* genotypes was greater in barley cultivar mixtures than in pure stands controls [37, 38]. Selection for complexity was more intense in random mixtures of barley cultivars than that from row mixtures, which predict less intense selection for complexity as autoinfection increases [39]. Distinctly of those observed in these random mixtures of sorghum lineages, aloinfection can predominate, considering the small size of both these plants and the plots [12]. Another interesting explanation is that, at larger spatial scale, *B. graminis* increased in complexity in the former German Democratic Republic, where barley mixtures were being grown on as much as 360,000 ha.

Different models have been proposed to describe the conditions in which complex races, able to develop on several components of the mixture, are selected and erode the resistance of the mixture. The development of more complex races in genotype mixtures observed in this study is in agreement with what was predicted by some authors, in their models on pathogen evolution in host mixtures [32, 40, 41]. The development of complex races of *C. sublineolum* in mixtures did not reduce however, the effectiveness of the mixture in reducing anthracnose severity in these different mixtures was evaluated. In artificially inoculated plots, the proportion of complex races was presented in a mixture depended on the environment, host genotypes included in the mixture and the genetic background of the complex race [34]. In a greenhouse experiment, attempt was made to dissociate genetic background from virulence of *Puccinia graminis* in

barley cultivar mixtures [42]. The author found strong selection for pathogen genotypes with virulence corresponding to three of the five resistance genes present in the multiline populations, even when pathogen genotypes more complex represented a minority and at low frequencies. Given that the same virulence was selected on the susceptible, like in this report, this selection may have been due more to fitness effects associated with specific combinations of virulence than to any selective effect of the multilines. Like described above, in Eastern Germany, after several years of extensive commercial use of barley cultivar mixtures, there was an increase in the relative, but not absolute, frequency of *B. graminis*, combining virulence to two of the three resistance genes most commonly used in the mixtures, and then, despite the relative increase in the frequency of complex races in the mixtures compared to pure stands, little loss of disease control would occur.

The results reported in this paper, like the other related, are all consistent with observations of natural ecosystems, in which complex races are the most frequent, but do not displace all other genotypes, and are not sufficiently fit to cause high levels of disease. Selection for increased fitness of complex races could occur over multiple seasons in agricultural systems, if a mixture with a small number of host components was to be grown continuously [38]. Most of the models that have been used studying evolutionary processes in the mixtures, which have incorporated that fitness costs associated with virulence are the only mechanisms to counter selection for increased virulence complexity in the pathogen. A review paper has described that the advantage to a complex race of being able to attack multiple host genotypes in a mixture is hypothesized to be counter to a reduction in fitness associated with lack of avirulence genes that correspond to the matching resistance genes [9]. So, according to this author, these models suggest that it will be difficult to prevent complex races from eventually dominating the pathogen population,

although the dominance of these complex pathotypes in these sorghum lineages mixtures has been characterized in this work. This author also reported that the process may be sufficiently slow that the pathogen population can be managed and that expected reduction in fitness of the complex races developed in some genotype mixtures as differences in competitive ability among races with different degrees of complexity has been demonstrated in *C. sublineolum* from sorghum anthracnose [43-45]. Finally, the protection given for the resistant sorghum lineages over these susceptible sorghum lineages at random mixtures to suppress considerable anthracnose severity shows the applicability of this strategy in disease control and gives hope to applications on large experimental areas within a wide sample collection to attempt a best description of a pathogen evolution in these experimental sorghum mixtures.

5. Conclusions

With the base of this biological screening test, it was possible to verify that the importance of the plants used in this work, which had many applications to evaluate the behavior of plant pathogens populations under disease control strategies was based upon cultivar mixtures approach. On this manner, in such case of study, anthracnose is the most prevalent disease in sorghum in Brazil, and several other places around the world, and actually in serious dangerous conservation status of natural enemies to this pathogens.

In fact, the authors' findings clearly indicate a tendency in the increase of virulence genes of the pathogens associated with the cultures in a mixture of genotypes and, conversely, a reduction of the diversity of pathotypes under the same conditions. Unlike the plots in mixtures, pure-plots strains reduced the number of virulence genes against all sorghum strains tested, and increased the diversity of pathotypes in general. This result is in agreement with the models tested in several pathosystems, in which there is a clear reduction of disease severity, with a concomitant

increase in the frequency of pathogenic races with high virulence.

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References

- [1] Ferreira, A. S., and Casela, C. R. 1986. "Pathogenic Breeds of *C. graminicola*, Causal Agent of Anthracnose in Sorghum (*Sorghum bicolor*)." *Fitopatologia Brasileira* 11: 83-7.
- [2] Casela, C. R., and Ferreira, A. S. 1987. "Reaction of Sorghum Genotypes to Seven Pathotypes of *Colletotrichum graminicola*, Causal Agent of Anthracnose." *Fitopatologia Brasileira* 12: 60-2.
- [3] Casela, C. R., Santos, F. G., and Ferreira, A. S. 2000. "Pathogenicity and Phenotypic Diversity Association of *Colletotrichum graminicola*, Causal Agent of Anthracnose in Sorghum." *Fitopatologia Brasileira* 25 (3): 517-21.
- [4] Garrett, K. A., and Mundt, C. C. 2000. "Host Diversity Can Reduce Potato Late Blight Severity for Focal and General Patterns of Primary Inoculum." *Phytopathology* 90: 1307-12.
- [5] Barrera, J. A. S., and Frederiksen, R. A. 1994. "Evaluation of Sorghum Hybrid Mixtures for Controlling Sorghum Leaf Blight." *Plant Disease* 78: 499-503.
- [6] Guimarães, F. B., Casela, C. R., Santos, F. G., and Ferreira, A. S. 1998. "Control of Sorghum Anthracnose through the Use of Mixtures of Cultivars." *Summa Phytopathologica* 24 (2): 131-5.
- [7] Ngugi, H. K., King, S. B., and Julian, A. M. 2001. "Simultaneous Temporal Progress of Sorghum Anthracnose and Leaf Blight in Crop Mixtures with Disparate Patterns." *Phytopathology* 91: 720-9.
- [8] Finckh, M. R., Gacek, E. S., Goyeau, H., Lannou, C., Merz, U., Mundt, C. C., Munk, L., Nadziak, J., Newton, A. C., De Vallavieille-Pope, C., and Wolfe, M. S. 2000. "Cereal Variety and Species Mixtures in Practice, with Emphasis on Disease Resistance." *Agronomie* 20: 813-37.

- [9] Mundt, C. C. 2002. "Use of Multiline Cultivars and Cultivar Mixtures for Disease Management." *Annual Review of Phytopathology* 40: 381-410.
- [10] Browning, J. A., and Frey, K. J. 1969. "Multiline Cultivars as a Means of Disease Control." *Annual Review of Phytopathology* 7: 355-81.
- [11] Wolfe, M. S. 1985. "The Current Status and Prospects of Multiline Cultivars and Variety Mixtures for Disease Resistance." *Annual Review of Phytopathology* 23: 251-73.
- [12] Garrett, K. A., and Mundt, C. C. 1999. "Epidemiology in Mixed Host Populations." *Phytopathology* 89: 984-90.
- [13] Vanderplank, J. E. 1968. *Disease Resistance in Plants*. New York: Academic Press.
- [14] Abbott, D. C., Burdon, J. J., Brown, A. H. D., Read, B. J., and Bittisnich, D. 2000. "The Incidence of Barley Scald in Cultivar Mixtures." *Australian Journal of Agricultural Research* 51: 355-60.
- [15] Newton, A. C., Guy, D. C., Nadziak, J., and Gacek, E. 2002. "The Effect of Inoculum Pressure, Germplasm Selection and Environment on Spring Barley Cultivar Mixtures Efficacy." *Euphytica* 125: 325-35.
- [16] Barrett, J. A. 1980. "Pathogen Evolution in Multilines and Variety Mixtures." *Z. Pflanzenkrankh* 87: 383-96.
- [17] Leonard, K. J., and Czocho, R. J. 1980. "Theory of Genetic Interactions among Populations of Plants and Their Parasites." *Annual Review of Phytopathology* 18: 237-58.
- [18] Mundt, C. C., and Browning, J. A. 1985. "Development of Crown Rust Epidemics in Genetically Diverse Oat Populations: Effect of Genotype Unit Area." *Phytopathology* 75: 607-10.
- [19] Mundt, C. C., and Leonard, K. J. 1986. "Analysis of Factors Affecting Disease Increase and Spread in Mixtures of Immune and Susceptible Plants in Computer-Simulated Epidemics." *Phytopathology* 76: 832-40.
- [20] Kiyosawa, S. 1989. "Breakdown of Blast Resistance in Relation to General Strategies of Resistance Gene Deployment to Prolong Effectiveness of Disease Resistance in Plants." In *Plant Disease Epidemiology: Genetics, Resistance and Management*, Vol. 2, edited by Leonard, K. J., and Fry, W. E. New York: McGraw-Hill, 251-83.
- [21] Marshall, D. R. 1989. "Modeling the Effects of Multiline Varieties on the Population Genetics of Plant Pathogens." In *Plant Disease Epidemiology: Genetics, Resistance and Management*, Vol. 2, edited by Leonard, K. J., and Fry, W. E. New York: McGraw-Hill, 81-8.
- [22] Cowger, C., and Mundt, C. C. 2002. "Effects of Wheat Cultivar Mixtures on Epidemic Progression of *Septoria tritici* Blotch and Pathogenicity of *Mycosphaerella graminicola*." *Phytopathology* 92: 617-23.
- [23] Torres, J. C., and Ventura, J. A. 1991. "A Program to Calculate Area and Volume below the Disease Progress Curve." *Fitopatologia Brasileira* 16: 52.
- [24] Cruz, C. D. 2001. *GENES—Software for Experimental Statistics in Genetics*. Viçosa, Brazil: UFV.
- [25] Cardwell, K. F. 1989. "Variation in Virulence of *Colletotrichum graminicola* (Ces.) Wils. and Competition between Monoconidial Isolates." Ph.D. thesis, College Station, Texas A&M University, Texas, USA.
- [26] Groth, J. V., and Roelfs, A. P. 1987. "The Concept and Measurement of Phenotypic Diversity in *Puccinia graminis* on Wheat." *Phytopathology* 77 (10): 1395-9.
- [27] Mundt, C. C., and Leonard, K. J. 1985. "Effect of Host Genotype Unit Area on Epidemic Development of Crown Rust Following Focal and General Inoculations of Mixtures of Immune and Susceptible Oat Plants." *Phytopathology* 75: 1141-5.
- [28] Cardwell, K. F., Collins, S. D., and Frederiksen, R. A. 1988. "Dilatory Resistance Character of Sorghum Hybrids as Measured by Area under Disease Progress Curve." *Biological and Cultural Tests* 3: 36-41.
- [29] Guimarães, F. B., Casela, C. R., Santos, F. G., Pereira, J. C. R., and Ferreira, A. S. 1999. "Evaluation of Resistance of Sorghum to Anthracnose Genotypes." *Summa Phytopathologica* 25 (4): 308-12.
- [30] Ross, W. M. 1965. "Yield of Grain Sorghum (*Sorghum vulgare* Pers.) Hybrids Alone and in Blends." *Crop Science* 5: 593-4.
- [31] Zhu, Y., Chen, H., Fan, J., Wang, Y., and Li, Y. 2000. "Genetic Diversity and Disease Control in Rice." *Nature* 406: 718-22.
- [32] Lannou, C. 2001. "Intrapathotype Diversity for Aggressiveness and Pathogen Evolution in Cultivar Mixtures." *Phytopathology* 91: 500-10.
- [33] Browning, J. A., and Frey, K. J. 1981. "The Multiline Concept in Theory and Practice." In *Strategies for the Control of Cereal Diseases*, edited by Jenkyn, J. F., and Plumb, R. T. Oxford: Blackwell, 37-46.
- [34] Dileone, J. A., and Mundt, C. C. 1994. "Effect of Wheat Cultivar Mixtures on Populations of *Puccinia striiformis* Races." *Plant Pathology* 43: 917-30.
- [35] Muller, K., McDermott, J. M., Wolfe, M. S., and Limpert, E. 1996. "Analysis of Diversity in Populations of Plant Pathogens: The Barley Powdery Mildew Pathogen across Europe." *European Journal of Plant Pathology* 102: 385-95.
- [36] Koizumi, S. 2001. *Rice Blast Control with Multilines in Japan*. Los Banos, Philippines: Inter. Rice Res. Inst., 143-57.
- [37] Munk, L. 1983. "Response of a Powdery Mildew Population to a Barley Variety Mixture." In *Durable*

- Resistance in Crops*, edited by Lamberti, F., Waller, J. M., and Van der Graaf, N. A. New York: Plenum, 105-7.
- [38] Chin, K. M., and Wolfe, M. S. 1984. "The Spread of *Erysiphe graminis* f. sp. *hordei* in Mixtures of Barley Varieties." *Plant Pathology* 33: 89-100.
- [39] Huang, R., Kranz, J., and Welz, H. G. 1994. "Selection of Pathotypes of *Erysiphe graminis* f. sp. *hordei* in Pure and Mixed Stands of Spring Barley." *Plant Pathology* 43: 458-70.
- [40] Lannou, C., and Mundt, C. C. 1996. "Evolution of a Pathogen Population in Host Mixtures. I. Study of the Simple Race-Complex Race Equilibrium." *Plant Pathology* 45: 440-53.
- [41] Lannou, C., and Mundt, C. C. 1997. "Evolution of a Pathogen Population in Host Mixtures: Rate of Emergence of Complex Races." *Theoretical Applied Genetics* 94: 991-9.
- [42] Kolmer, J. A. 1995. "Selection of *Puccinia recondita* f. sp. *tritici* Virulence Phenotypes in Three Multilines of Thatcher Wheat Lineages near Isogenic for Leaf Rust Resistance Genes." *Canadian Journal of Botany* 73: 1081-8.
- [43] Cardwell, K. F., Hepperly, P. R., and Frederiksen, R. A. 1989. "Pathotypes of *Colletotrichum sublineolum* and Seed Transmission of Sorghum Anthracnose." *Plant Disease* 73: 255-7.
- [44] Casela, C. R., Ferreira, A. S., and Santos, F. G. 2001. "Differences in Competitive Ability among Races of *Colletotrichum graminicola* in Mixtures." *Fitopatologia Brasileira* 26: 217-9.
- [45] Souza, B. O., Casela, C. R., Nunes, J. A., and Castro, H. A. 2013. "Management of Sorghum Anthracnose through Diversification of Genetic Resistance in Host Population." *Tropical Plant Pathology* 38 (1): 20-7.