CARTA AO EDITOR / *LETTER TO THE EDITOR*

The phaeosphaeria leaf spot of maize in Brazil: evidences of a new etiological agent?

A leaf spot disease, described as phaeosphaeria leaf spot, has been observed causing severe damage in all maize-growing areas of Brazil since 1982 (Fantin, 1994). The causal organism of this disease was initially described in the USA as *Phaeosphaeria maydis* (Henn.) Rane, Payak & Renfro (sin. *Sphaerulinia maydis* Henn.), anamorph *Phyllosticta* sp. (Rane *et al.*, 1966). Doubts regarding the etiology of this disease have resulted in several attempts to perform Koch´s postulates in order to identify the real causal agent of this disease in Brazilian conditions (Fantin & Balmer, 1997; Paccola–Meirelles *et al.*, 2001; Amaral *et al*., 2005).

A recent publication by Oliveira *et al.* (2004, Revista Brasileira de Milho e Sorgo 3:343–356) postulates a species of *Sclerophthora* as the probable agent of the disease known in Brazil as phaeosphaeria leaf spot of maize. The quality of the information concerning a disease of such importance has to be examined very carefully, as misleading information can cause confusion and also delay progress in key research areas for the development of successful strategies to manage this disease. Thus, as plant pathologist working on maize diseases over the last twenty years, we thought it our duty to draw the scientific community's attention to some points concerning the results described by Oliveira *et al.* (2004) which could invalidate the whole concept of a new etiological agent for this disease, as postulated by those authors. In this regard, the above mentioned paper shows a set of photographs whose interpretation provokes heavy doubts. Critical points regarding the illustrated structures are discussed in this letter.

Figure 1 on page 346 of the paper shows typical symptoms of the phaeosphaeria leaf spot of maize. Lesions are round or oval, varying in diameter from 0.3 to 1.0 cm. Lesions may also coalesce and become irregular shaped (Fernandes & Oliveira, 1997; Parentoni *et al.*, 1994). These symptoms are clearly different from typical symptoms caused by the two known species of the genus *Sclerophthora* causing downy mildew on maize. *Sclerophthora macrospora* (Sacc.) Thirum., C.G. Shaw & Naras. causes an excessive tillering, rolling, twisting of the upper leaves, and a partial or complete proliferation of the tassel, which continues until tassel resembles a mass of leafy structures. These modified leaves, like inflorescences, are described as "crazy top" (Shurtleff, 1986). Typical symptoms of the brown stripe downy mildew, caused by *Sclerophthora rayssiae* R.G. Kenneth, Koltin & I. Wahl are characterized by the development of narrow chlorotic or yellowish stripes, 3-7 mm wide, with well defined margins and delimited by the veins. The stripes later become reddish to purple. Lateral development of lesions causes severe stripping and blotching (Shurtleff, 1986). Symptoms described in Figure 1 are also completely different from the symptoms of maize downy mildews, caused by *Sclerospora graminicola* (Sacc.) J. Schröt., *Peronosclerospora sorghi* (W. Weston & Uppal) C.G. Shaw, *P. sacchari* (T. Miyake) C.G. Shaw, *P. philippinensis* (W. Weston) C.G. Shaw, *P. maydis* (Racib.) C.G. Shaw, and *P. spontanea* (W. Weston) C.G. Shaw. A common feature of all these downy mildews is the development of leaf stripes or leaf streaks, extending the length of the leaves (Shurtleff, 1986). It is, therefore, highly improbable to have a *Sclerophthora* organism causing symptoms on maize leaves that follow a completely different pattern, not only from those caused by the genus *Sclerophthora*, but also from those caused by all of the other causal agents of downy mildews on maize.

Oliveira *et al.* (2004) showed in Figure 2, Page 348, two structures described by the authors as sporangia of *Sclerophthora*. These structures are without any doubt urediniospores of a rust fungus. The spores are echinulate with prominent equatorial germ pores, very similar to urediniospores of *Puccinia sorghi*, the causal organism of the common rust of maize, although spore size of all the three species causing rust in maize are within the range of spore dimension (Shurtleff, 1986). A round pore on one of the spores is a typical germ pore of an urediniospore and not an operculum, as described by the authors. Typical sporangia of Peronosporaceae are smooth-walled and larger than the structure shown in Figure 2, as confirmed by F. Ferreira, Y. Hiratsuka and R. Taber (personal communications, 2005). For example, sporangia of the species *Sclerophthora macrospora* measure 60-100 x 43-64 µm, and those of *S. rayssiae* 29-66.5 x 18.5-26 µm (Payak & Renfro, 1967).

Structures described in Figure 3, Page 349, are no zoospores, do not appear to have flagella and are definitely not kidney-shaped. Strands are more like extraneous materials, perhaps deposited as a result of preparatory fixation for scanning, as interpreted by F. Ferreira and R. Taber (Personal communications).

The structure in Figure 4, Page 350, described as "germinating oospores", cannot be interpreted as such. First of all, a photograph of an oospore, if this structure could be taken as such, using regular light microscope and stained to show its thick wall would have been more clarifying or diagnostic and really indicative of the taxonomic position of the organism (R. Taber, personal communications). Furthermore, oospores are produced and found scattered in the leaf mesophyll or under the stomata (Shurtleff, 1986; Payak & Renfro, 1967). In the case of the genus *Sclerophthora*, oospores are structures of survival or resistance that are released to the soil where they will produce zoospores that penetrate the host tissues and produce systemically infected plants (Shurtleff, 1986; Craig, 2000). The chances of finding germinating oospores on a leaf surface are, therefore, very low.

The interpretations of Figure 6A and B on page 352 are questionable and the authors themselves are in doubt. The description of structures in Figure 6A as an antheridium and an oogonium is not diagnostic enough to be accepted as such. The same can be said about Figure 6B. The authors consider this complex structure as "many oogonia x antheridium?" The correct description should be "many antheridia x oogonia", a detail that was not observed by the reviewers of the paper. Another important comment about Figures 6A-B is the fact that they are described as the same structures, although there is strong evidence that they are, in fact, different. Based on the values of magnification provided by the authors, the real diameter of the structure in Figure 6A is 72 µm. On the other hand, the real size of the structure in Figure 6B, again based on the magnification provided by the authors, is between 9 and 12 µm, which leads to the conclusion that structures shown in Figures 6A and B cannot be considered as being of the same nature. Urediniospores with 9 µm of diameter are, apparently, dehydrated as a consequence of preparation of material for scanning (F. Ferreira, Y. Hiratsuka, R. Taber; personal communications).

Figures 7 and 8 on page 353 show structures under the stereomicroscope and optical microscope, respectively, which are described by the authors as sporangia of *Sclerophthora*. These structures can not be accepted as sporangia for several reasons: 1) based on the magnification provided by the author (80X), the structures presented in Figure 7 measure 62 µm on average; 2) based on the magnification provided by the authors (400X), structures shown in Figure 8 measure 125 µm, which means that they are twice as large as structures shown in Figure 7, which indicates that they are of different nature or, in other words, they represent different organisms (F. Ferreira, personal communication); 3) the structures described as sporangia by the authors in Figure 7 give no indication of the presence of sporangiophores, which reinforces the argument that these are not, in fact, sporangia; 4) the supposed sporangia in Figure 8 have a globose shape while real sporangia of the genus *Sclerophthora* are lemon-shaped (Payak & Renfro, 1967; Shurtleff, 1986); 5) finally, the so-called sporangia in Figure 8 have a smooth surface and are much bigger than the spores in Figure 2 on page 348, which indicates that they belong to different organisms and none of them are, in fact, sporangia of *Sclerophthora* (according to Y. Hiratsuka, personal communication). The fact that all structures described by the authors as sporangia cannot be accepted as such, rises a further question: if there are no sporangia, what are the structures described as zoospores in Figure 3 and where do they come from?

Finally, it is important to mention that the authors have made no inoculation with the organisms identified as *Sclerophthora* to confirm their pathogenicity and reproduce the symptoms of the disease on maize leaves. Koch's postulates were not followed to verify their hypothesis that this supposed *Sclerophthora* was the cause of the phaeosphaeria leaf spot. Considering the fact that this fungus is an obligate parasite, the authors should have followed the steps as indicated in Agrios (2004). The fact that Koch's rules were not performed shows a complete lack of any scientific evidence supporting the suggestion that *Sclerophthora* sp. could be the causal organism of the phaeosphaeria leaf spot in Brazil.

Our objective in sharing these ideas with the scientific community is to avoid more confusion in a topic that is still under discussion, such as the etiological agent of the maize disease called Phaeospheria Leaf Spot. We think that in this way we may help to avoid spreading an idea that clearly is not based on any scientific evidence. In summary, Oliveira *et al.* (2004) did not show any positive results and therefore this paper cannot be considered as scientifically valid.

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