Mycosphaerella graminicola, the causal agent of septoria tritici blotch (STB), is a major wheat disease in Europe. Disease control currently depends on fungicide application and requires an annual fungicide input of approximately 600 M€. In addition several breeding companies and institutes have identified resistance in their breeding material and try to introgress these resistance genes into commercial cultivars. Despite these efforts M. graminicola continues to be a threat, as resistance in wheat is often not durable as it is governed by a gene-for-gene relationship and fungicide resistance can arise and increase quickly. The later was recently exemplified by the rapid spread of resistant isolates to one of the most important classes of fungicides, the strobilurins. Consequently, there is a continuous need for the development and evaluation of new fungicides and new resistant breeding material. However, the current visual scoring in evaluation procedures is far from optimal as visual symptoms tend to be variable, are difficult to quantify, their scoring is subjective, time consuming and therefore expensive. Ideally one would like to monitor and quantify the pathogen biomass over time in different hosts and treatments. We developed a new screening platform, called SeptoriaScreen, that aims to do this. SeptoriaScreen is based on high throughput fluorescence imaging (HTFI) and enables real-time, detailed and non-destructive studies of the M. graminicola -wheat interaction using GFP and RFP tagged M. graminicola transformants of isolate IPO323. The GFP and RFP reporter strains with the reporter under the control of the constitutive GPD or TOX promoter were tested for their fluorescence under in vitro and in planta conditions using the laser induced high resolution fluorescence imaging platform. The strains with the highest fluorescence were selected and the colonization process of wheat was visualized and quantified using custom made software and compared directly to the visual symptoms on high resolution color images. This platform has been applied to monitor resistance levels in wheat genotypes and in efficacy screening of fungicides. We quantified the effect of different wheat resistances and the efficacy of fungicides such as prothioconazole and azoxystrobin. SeptoriaScreen allows the quantification of the pathogen during the complete infection cycle. Starting directly after inoculation until the leaves become fully necrotic and filled with pycnidia. SeptoriaScreen opens new windows to study the infection process and allows fast, detailed and sensitive quantification of the pathogen.