Results and Discussion

In Kenya, B. holstii was found in the highlands of the Rift Valley and northern slope of Mt Kenya in an elevation range of 2000 - 3000m (Fig. 1A), where wheat is normally grown. Aecial infections were observed in August in the Mt Kenya area. In Ethiopia, B. holstii was found primarily in North Shewa Zone, with a similar range of elevations (Fig. 1B). Aecial infections (Fig. 2) were observed from June to December with highest infection intensity in August to October.

For the majority of samples, aeciospore viability was lost. Using relatively fresh samples collected in North Shewa in 2012 and 2014, inoculations resulted in stem rust infections on Line E, Prolific, Hiproly, and Marvelous (Table 1).

DNA assays confirmed the presence of P. graminis in these samples (Table 2). Inoculations and DNA assays did not detect the presence of P. striiformis. While it is likely that the rust pathogen infecting Line E, Hiproly, and Prolific is P. graminis f. sp. secalis (Pgs), inoculation and DNA assays did not provide sufficient resolution to distinguish Pgs from P. graminis f. sp. tritici (Pgt). Stem rust infections observed on Marvelous were assumed to be P. graminis f. sp. avenae. Experiments are in progress to characterize isolates derived from these samples, and to determine if other rust fungi are present. Based on these preliminary data, we conclude that P. graminis completes its sexual cycle in Ethiopia. The contribution of the sexual cycle to the observed variation within the Pgt population in the region remains unclear.

Materials and Methods

Field surveys of B. holstii distributions and collections of aecial infections.

Field surveys conducted since 2008 in Kenya and 2009 in Ethiopia, focused on areas where small grain cereals, primarily wheat and barley, are grown. B. holstii herbarium collections were used to locate plants in Ethiopia. Aecial infections were collected, air-dried, shipped to USA, and stored at 4°C until use in inoculation experiments.

Inoculation on cereals and grasses.

A series of cereal species, termed identification series, and consisted of wheat (Triticum aestivum cv. Morocco and Line E), barley (Hordeum vulgare cv. Hiproly), rye (Secale cereale cv. Marvelous), and oat (Avena sativa cv. Marvelous), was used to isolate different formae speciales (f. sp.) of P. graminis on cereals from infected barberry leaves. Infected barley leaves were suspended over seedling plants (7 to 10 days post-planting) and incubated in a dew chamber for 48 hrs. When a sample was limited, aeciospores were collected, suspended in mineral oil, and spray-inoculated to seedlings. Two incubation temperature regimes, 18-20°C and 12-15°C, were used in the infection period.

DNA Assay.

Ten individual aecia from each sample were used for DNA extractions. DNA was extracted following a modified protocol of the CTAB method. A SmartCycler was used to perform real-time PCR reactions (Barnes & Szabo 2007). Three specific probes and primers were used for the real-time PCR reactions. One set was used to test for P. graminis (Pgr) and two sets were used to test for P. striiformis (PsFAM2 and PsFAM4).

Acknowledgements

This research is funded by USDA-ARS and the Durable Rust Resistance of Wheat (Cornell). The authors thank Sam Gale, Lucy Wanschura, Kim P. Nguyen, and Melissa Carter for their technical assistance.

References