Bhutan Wheat Rust Surveys - April 2012 - Summary Report

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Summary

Cereal rust surveys were undertaken by the National Plant Protection Centre, Department of Agriculture, Ministry of Agriculture and Forest, Semtokha, Thimpu, Bhutan and the Research and Development Centre, Bajo, Department of Agriculture, Ministry of Agriculture and Forest, Bajo, Wangduephodrang in collaboration with the Durable Rust Resistance in Wheat (DRRW) project during the period 17-21st April 2012. Surveys were undertaken in the wheat/barley/oat growing areas of five Dzongkhag's in western Bhutan (Haa, Paro, Punakha, Gasa, Wangdi Phodrang). A total of 26 individual sites were visited, with several cereal fields surveyed for diseases at each location using standard BGRI survey methodology. Surveys were conducted in agricultural areas, primarily along river valleys. Elevations of sites visited ranged 1200m to 2700m, with bread wheat, barley and oats included in the survey. The majority of the cereal crops surveyed were at the flowering-milk growth stage, although at higher elevations crops were observed at earlier growth stages (tillering). Follow up surveys are planned by NPPC staff to check for the presence of stem rust at later growth stages. The primary focus of the surveys was to assess the status of cereal rusts in Bhutan and to monitor the potential spread of new virulent races of stem rust (Ug99 race lineage) into the South Asian region. The role of Berberis sp. as a potential alternate host for cereal rusts (stem and stripe rust) was also investigated. Berberis sp. bushes in close proximity to cereal crops and along the roadside were checked for aecial infections along the survey route.

No stem rust was observed at any of the survey sites visited. Individual pustules with unusual morphology indicative of possible stem rust infections were collected at several sites, but subsequent microscopy at the NPPC laboratory indicated that all such pustules were actually leaf rust. Symptoms indicative of stem rust on a grass species were also found to exhibit spore morphology typical of leaf rust following microscopy analysis.

Stripe (yellow) rust was the most widespread cereal rust, being observed at 13 out of the 26 sites visited. Highest incidence and severity of stripe rust was observed in Punakha Dzongkhag. Four of the sites visited in Punakha exhibited moderate to high severity of disease. The commonly grown cultivar "Sonalika" was susceptible to stripe rust. Both bread wheat and barley were infected with stripe rust. Low incidence and severity of stripe rust was also observed in the southern part of Paro Dzongkhag.

Leaf rust was observed at 11 out of the 26 sites visited. Distribution of the disease essentially mirrored that observed for stripe rust, with most observations occurring in Punakha Dzongkhag. Leaf rust was also recorded at two sites in Paro Dzongkhag. At all sites, except Wangdue research station, only low incidence and severity of leaf rust were recorded on the survey. However, increases in both incidence and severity of disease may occur as temperatures rise. The commonly grown cultivar Sonalika was considered moderately susceptible to susceptible to leaf rust.

Berberis spp. were common in the survey areas, present along roadsides and in close proximity to cereal crops. Identification to species level was not possible, but it was apparent that several distinct species of *Berberis* were present (possibly at least five distinct species?). *Berberis* spp. infected with rust aecia were observed at three distinct locations – one location in southern Punakha (Omtekha village) and two locations in Paro (Shabdrujedingkha and Susuna) . At each location, several *Berberis* bushes were found to be heavily infected with aecia. All infected *Berberis* had broad-leaf morphology, but at least two different species were considered to be susceptible to aecial infections. None of the narrow-leafed *Berberis* sp. examined had any signs of aecial infection. Two of the infected sites had stripe and leaf rust in relatively close proximity to the infected *Berberis*, whereas at the third site no cereal rusts were observed at the location. Grass species infected with rust sp. were found growing next to infected *Berberis* at one location (Omtekha).

Samples of cereal rusts (wheat stripe rust, barley stripe rust, wheat leaf rust and barley leaf rust) were collected from all infected sites. Samples of *Berberis* leaves infected with aecia were also collected from infected sites. Samples were sent under permit to international rust laboratories in the USA, Denmark and India for subsequent race analysis.

Background

Wheat, barley and oats are all minor cereal crops in Bhutan compared to rice and maize. Recent official agricultural statistics (2009-10) estimate the wheat area to be in the order of 2000-3000 ha and the estimated barley area to be about 2000ha. However, recent Bhutanese government policy decisions have placed more emphasis on supporting domestic production. The importance of the Himalaya region in terms of rust epidemiology has been recognized since the pioneering rust work of Prof K.C. Metha in India in the early 1900's.

Wheat production in Bhutan is concentrated in the warm temperate (high altitude) zone and the Dry sub-tropical (medium altitude) zone (Table 1). Important Dzongkhags for production include; Paro,Haa, Thimpu, Gasa, Bumthang, Wangdiphodrang, Punakha, Trongsa, Tashigang, Mongar and Lhuentse. Only 3 improved spring cultivars have been released in Bhutan, namely sonalika (1988), Bajoka 1 (1991), Bajoka 2 (1994), but older landraces are also cultivated.

Little is known about the current status of wheat diseases, and rusts in particular, in Bhutan. Nagarajan and Joshi (1985) mentions a paucity of information relating to rust pathotypes in Nepal, Bhutan and Bangladesh, but considered that many of the leaf and stripe rust races would be common throughout South Asia. Mann & Hobbs (1988) report substantial losses to stripe rust in 1985 and 1986 in the Punakha Valley and they considered this, at the time or reporting, to be the only disease in Bhutan known to cause serious epidemics in certain years. They also report the occurrence of leaf rust, which under normal conditions appears too late to cause any substantial losses. Although they did not rule out the possibility of occurrence of leaf rust epidemics in the Wangdi Punakha Valley if conditions were favourable. Classical rust studies undertaken in India have shown the importance of the Himalaya foothills as primary infection sources for both stripe rust and leaf rust (work of KC Metha cited by Nagarajan and Joshi, 1985). Over summering of rusts throughout the Himalaya region is also well documented. The current view prevailing in India is that the Himalaya region has hardly any effect in terms of dispersal of stem rust and the principal source of infection comes from the Nilgiri Hills in the South. Although it must be noted that globally stem rust is a disease that has been largely controlled for over three decades, so current epidemiology information is lacking in most regions. For both stripe and leaf rust the Himalaya region is considered a primary source of infection for the high production plain zones.

Berberis spp. are common throughout the Himalaya, but there is no conclusive evidence for a functioning role as alternate hosts for stem rust (Nagarajan and Joshi, 1985). *Berberis* was recently found to be a functional alternate host for stripe rust in North America (Jin et al. 2010), but no studies have yet been reported in the Himalaya region investigating the potential role of *Berberis* spp. as alternate hosts for stripe rust. Data from the National Biodiversity Centre, Bhutan indicates that at least 14 *Berberis* spp. are present in Bhutan (Table 2). At present, no information is available on either the susceptibility of these species to wheat rusts or their potential to act as a functional alternate host.

Despite the current period of remission for stem rust, new emerging races in Africa raise the potential for the disease to threaten global wheat crops. Historically stem rust has been the most feared wheat disease, capable of causing periodic severe devastation across all continents and in all areas where wheat is grown. There is a solid foundation behind this fear as an apparently healthy crop only three weeks away from harvest could be reduced to nothing more than a tangle of black stems and shrivelled grain by harvest. Under suitable conditions, yield losses of 70% or even complete crop loss are possible. In the mid 1950's over 40% of the North American spring wheat crop was lost to devastating stem rust epidemics (Leonard, 2001). These devastating losses were the result of the emergence of a new stem rust race named 15B, which overcame the genetic resistance in widely grown wheat cultivars at the time.

Since the epidemics of the 1950's, widespread use of resistant wheat cultivars worldwide has reduced the threat of stem rust to the extent that it is not a significant factor in wheat production. By the mid 1990's, stem rust was largely considered to be a disease under control (e.g., Roelfs et al., 1992). However, with the identification of a new virulent stem rust race (TTKSK), popularly named Ug99, in the wheat fields of Uganda in 1999 (Pretorius et al., 2000), that perspective has now changed. An estimated 80% of all global commercial wheat cultivars are considered susceptible to Ug99 or variants. As a result, stem rust is now very firmly back on the agenda of wheat scientists worldwide.

Ug99 is the only known race of stem rust that has virulence for the important stem rust resistance gene *Sr31*, a unique characteristic that facilitated its original identification. However, in addition it also shows virulence to most of the stem rust resistance genes originating from wheat, plus virulence to gene *Sr38* of alien origin. This combination of virulence is what makes Ug99 unique and why it is considered a potential major threat to global wheat production. The pathogen is continuing to change, resulting in variants that exhibit differing virulence and render further *Sr* genes ineffective. Seven variants are now recognized in the Ug99 race lineage, all very closely related. Acquired virulence to additional important Sr genes, notably *Sr24* and *Sr36*, is known from Kenya (*Sr24*, *Sr36* variants), Ethiopia (*Sr24* variant) and South Africa (*Sr24* variant). Race TTKST (carrying combined *Sr31* + *Sr24* virulence), caused epidemics in Kenya in 2007. Several variants pose an increased potential threat compared to the original Ug99 pathotype.

Stem rust is highly mobile, capable of travelling long distances via air-borne movements or accidental human-borne transmission. Since first detection, the original Ug99 pathotype (race TTKSK) has moved across East Africa (Uganda, Kenya, Ethiopia, Sudan), crossed into Yemen and had reached Iran by 2007. Known movements of TTKSK have most likely been via regional airflows. New variants in the Ug99 lineage are also increasing in geographical range, and likely to show similar long-distance movements. Evidence from wind models and circumstantial evidence of previous wheat rust race appearance (e.g., *Yr9* virulence), indicates there is a high possibility of continued movement of Ug99 lineage races into the South Asia region.

The emergence of the Ug99 lineage of stem rust in East Africa has prompted a global and concerted effort by wheat scientists to try and mitigate the threat posed. Nobel laureate Dr N.E. Borlaug was at the forefront of efforts to raise the alarm surrounding the potential threat of Ug99, convening an expert panel that published an assessment report in 2005 (CIMMYT, 2005). Following on from the 2005 expert panel assessment, an international global consortium termed the Borlaug Global Rust

Initiative (BGRI) (http://www.globalrust.org/) has been formed bringing together institutions interested in the mitigation of wheat rust diseases.

A key component of the global efforts to address the emerging threat posed by stem rust is effective monitoring and surveillance of the pathogen. As a result, a Global Cereal Rust Monitoring System has been established under the Durable Rust Resistance in Wheat (DRRW) project. National surveys are an essential part of this monitoring system and in this context initial surveys were undertaken by the National Plant Protection Centre, Bhutan and the Research and Development Centre, Bajo, Wangduephodrang in collaboration with DRRW project scientists (Cornell University, CIMMYT & PBI, Sydney University) throughout wheat growing areas of western Bhutan in April 2012. The aim is to develop a comprehensive knowledge base on the current rust situation in South Asia, to monitor the changes that are occurring, and to gain an in-depth understanding of how wheat rust pathogens migrate within South Asia. All monitoring and surveillance information is being disseminated through the Rust Spore.org web portal hosted by CIMMYT (See http://rustspore.cimmyt.org/).

Survey Report

A survey team consisting of personnel from the National Plant Protection Centre, Department of Agriculture, Ministry of Agriculture and Forest, Semtokha, Thimphu, Bhutan; the Research and Development Centre, Bajo, Department of Agriculture, Ministry of Agriculture and Forest, Bajo, Wangduephodrang and DRRW project scientists undertook surveys for wheat rust in the wheat/barley/oat growing areas of western Bhutan during the period 17-21st April 2012. A survey route was chosen to cover areas planted to wheat or other cereals in the Dzongkhags of Haa, Paro, Punakha, Gasa, Wangdi Phodrang . Surveys were undertaken using standard BGRI methodology in the Punakha, Paro and Haa river valleys. A range of different ago-ecologies were visited with survey sites ranging in elevation from 1200-2700m. A total of 26 individual locations were visited with several cereal crop fields inspected at each location. Maturity of the crops varied but the majority were at the flowering-milk growth stage, although at higher elevations crops were observed at earlier growth stages (tillering). In all areas visited *Berberis* spp. were examined for signs of aecial infections. Any *Berberis* in close proximity to cereal growing areas. All survey locations and infected *Berberis* spp. were geo-referenced using GPS.

In addition to the generation of disease data, an additional objective of the survey was the capacity building of Bhutanese scientists in survey techniques and cereal disease identification. Nine Bhutanese scientists participated in the survey. Standardized BGRI field survey techniques were demonstrated, including; the use of standardized field survey forms, GPS, smartphone-enabled field data capture, and sample collection. Training in disease identification was facilitated through the presence of expert pathologist, Prof. Park (University of Sydney, Australia).

Survey site details and survey results are summarized in Table 3. Stem rust (*Puccinia graminis*) was not recorded at any of the locations visited. Isolated, individual pustules exhibiting unusual morphology were observed at a couple of sites in the Punakha Valley and a grass sp. was observed with symptoms characteristic of stem rust underneath an infected *Berberis* sp. bush at Omtekha village. In all cases, subsequent microscopic observation of spore morphology revealed only circular spore structures typical of leaf rust.

Stripe rust (*Puccinia striiformis*) was the most widely observed rust pathogen, with the highest incidence and severity. Stripe rust was observed at 13 out of the 26 sites visited (Map 1). Highest incidence and severity of stripe rust was observed in Punakha Dzongkhag (photo 1). Four of the sites visited in Punakha exhibited moderate to high severity of disease. The commonly grown cultivar "Sonalika" was susceptible to stripe rust. Both bread wheat and barley were infected with stripe rust. Barley infected with stripe rust was observed at two locations - one in the Punakha Valley and one in the Paro Valley (Map 1). Low incidence and severity of stripe rust was also observed in the southern part of Paro Dzongkhag. At two locations, stripe rust infected bread wheat was in close proximity (<0.5km) to *Berberis* spp. infected with aecia.

Leaf rust (*Puccinia triticina*) distribution closely matched that observed for stripe rust, but incidence and severity was much lower. Leaf rust was observed at 11 out of the 26 sites visited (Map 2). Most observations occurred in Punakha Dzongkhag. Leaf rust was also recorded at two sites in Paro Dzongkhag. At all sites, except Wangdue research station, only low incidence and severity of leaf rust were recorded on the survey. However, increases in both incidence and severity of disease may occur as temperatures rise and the crops mature. The commonly grown cultivar Sonalika was considered moderately susceptible to susceptible to leaf rust.

Berberis spp. were extremely common along the survey route and at the survey locations. It was considered that at least 4 (possibly 5) different species were observed on the survey (Photo 2). Two very distinct phenologies were apparent: broad-leaved *Berberis* and narrow-leaved *Berberis*. Heavy aecial infections were observed at three locations – one in the southern Punakha Valley (Omtekha village), two in the mid to southern Paro Valley (Map 3, Shabdrujedingkha and Susuna). Only broad-leaved *Berberis* spp. were observed to be infected with aecia. No infections were observed on any of the narrow-leaved species. Based on leaf morphology it is possible that at least two different *Berberis* spp. were susceptible to aecial infection (Photo 3). At one location in the Punakha Valley, a grass infected with possible stem rust symptoms was growing underneath an infected *Berberis* bush, although subsequent microscopy revealed only round spores usually typical of leaf rust. No other infected grasses were observed in close proximity to infected *Berberis*. Bread wheat infected with stripe rust was observed in relative close proximity (<0.5 km) to infected *Berberis* at two locations.

Samples were collected of all cereal rusts on the survey. In total approximately 30 stripe rust and leaf rust samples were collected. In addition, several samples of leaf rust were collected from different grass species. Samples of infected *Berberis* leaves were also collected. Rust samples were air-dried and sent under permit to the Global Rust Reference Centre in Denmark (Stripe rust), the Flowerdale Research Laboratory, Shimla, India (Stripe rust, Leaf rust, Rust on grasses) and Fort Dietrich (Cereals Disease Lab), USA (*Berberis* leaves, Leaf rust). Duplicate samples of stripe and leaf rust were sent to the appropriate labs. Race analysis will be undertaken at the respective laboratories on viable samples and data reported upon completion of the analysis. Assuming viable samples are available for analysis, this should provide the first documented information on which stripe and leaf rust pathotypes are present in Bhutan. Single pustule samples of stripe, leaf rust and individual aecial cups were also collected and stored in ethanol for subsequent DNA analysis at PBI, University of Sydney.

Other diseases: In the course of the survey, additional diseases of cereal crops were observed. Smuts were undoubtedly the most widespread diseases observed, present in all areas visited. Loose smut (*Ustilago tritici*) on oats was prevalent in many of the oat crops surveyed. Incidence as high as 15-20% was observed in several fields. Covered smut (*Ustilago hordei*) on barley was also observed at several sites, but at generally low incidence (<5%). Noteworthy was the observation of flag smut (*Urocystis agropyri*) on bread wheat at three distinct locations (Map 4). Only isolated infections were observed at each location, but this may constitute the first recorded occurrence of the disease in

Bhutan. Cephalosporium streak was observed at several locations in barley crops. Viral diseases were also observed - symptoms of Barley yellow Dwarf virus, and a second viral-like disease of unidentified aetiology.

Conclusions

The current surveys provided a valuable update on the situation regarding wheat rusts and other cereal diseases in Bhutan. Despite minor crop status of wheat, barley and oats sufficient area is planted to these cereals to permit a significant build up of rust inoculum and to play a potentially important role in disease epidemiology for the wider South Asian region. Under increased and intensified wheat production, consideration for control of a range of cereal diseases (including rusts) should be undertaken as a high priority. The widespread distribution of Berberis spp., including species that are susceptible to aecial infections, merits further investigation to determine if there is an y functional role as an alternate host for either stem or stripe rust.

No stem rust was observed at of the survey locations, although the possibility of occurrence cannot be excluded as temperatures rise and the crops mature. Additional surveys later in the season would be recommended to check for the presence of the disease. Any observed infections of stem rust would be a high priority for sampling and subsequent race analysis. Both stripe and leaf rust were widespread in the areas surveyed. Incidence and severity of stripe rust was higher, particularly in the Punakha Valley. In the areas with highest stripe rust pressure, yield losses of up to about 20% might be expected. Negligible losses from leaf rust would be expected, even if the disease increases as the season progresses. The commonly grown cultivar Sonalika was susceptible to both rust diseases, release and promotion of a diverse set of new improved cultivars with good rust resistance is seen as a very high priority.

Smuts (loose smut, covered smut and flag smut) were also widespread and consideration should be given to effective control. Incidence and severity of loose smut on oats in particular was very high in certain areas. All of the smuts can be controlled by effective seed treatment. The presence of flag smut was noteworthy as this may well constitute the first confirmed instance of the disease in Bhutan. The disease is known from Nepal and India, so occurrence in Bhutan is not totally unexpected. Flag smut can be a serious disease on wheat, it is both seed borne and persists in the soil giving rise to soil borne infections. Effective seed treatment, use of resistant cultivars and long rotations or fallow periods (the pathogen can persist for up to three years in the soil) are all recommended control practices.

The current surveys provided an indication of the status of important cereal diseases in Bhutan and subsequent rust race analysis should provide a good indication of which pathotypes predominate. However, in light of the continually changing nature of rusts and the known occurrence of new virulent races, it is considered important that cereal rust surveillance is undertaken on a regular basis in the future. Also critical is the linkage between pathology information and breeding or cultivar release programs. Ideally, any important changes in virulence should be accompanied by the release of cultivars with adequate resistance. Good control of cereal rusts in Bhutan, through the use of durably resistant cultivars, would have significant benefits for the wider region.

Acknowledgments

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Agro-ecological zone	Dzongkhags	Altitude (m)	Area (ha)	Production (t)	Productivity (t/ha)
Warm	Paro, Haa,	>1800m	1129	2440	2.1
Temperate	Thimpu, Gasa,				
(high altitude)	Bumthang				
zone					
Dry Sub-	Wangdiphodrang,	1200-1800	845	1972	2.3
Tropical	Punakha, Trongsa,				
(medium	Tashigang,				
altitude) zone	Mongar, Lhuentse				
Humid Sub-	Tsirang, Dagana,	600-1200	173	305	1.7
Tropical (mid	Zhemgang,				
altitude) zone	Chukha,				
	Pemagatshel,				
	Trashiyangtse				
Wet Sub-	Samdrupjongkhar,	<600	78	156	2.0
Tropical (low	Samtse, Sarpang				
altitude) zone					
Total			2225	4873	2.1

Table 1. Wheat harvested area and production in 2010 (Bhutan Department of Agriculture, 2011)

Berberis Species	Source
Species: Berberis angulosa	Species Diversity of Bhutan, National Biodiversity
	Center, Bhutan (http://portal.nbc.gov.bt/portalBhutan)
Species: Berberis aristata	Species Diversity of Bhutan, National Biodiversity
	Center, Bhutan (http://portal.nbc.gov.bt/portalBhutan
Species: Berberis asiatica	Species Diversity of Bhutan, National Biodiversity
	Center, Bhutan (http://portal.nbc.gov.bt/portalBhutan
Species: Berberis beesiana	Species Diversity of Bhutan, National Biodiversity
	Center, Bhutan (http://portal.nbc.gov.bt/portalBhutan
Species: Berberis concinna	Species Diversity of Bhutan, National Biodiversity
	Center, Bhutan (http://portal.nbc.gov.bt/portalBhutan
Species: Berberis cooperi	Species Diversity of Bhutan, National Biodiversity
	Center, Bhutan (http://portal.nbc.gov.bt/portalBhutan
Species: Berberis griffithiana	Species Diversity of Bhutan, National Biodiversity
	Center, Bhutan (http://portal.nbc.gov.bt/portalBhutan
Species: Berberis hookeri	Species Diversity of Bhutan, National Biodiversity
	Center, Bhutan (http://portal.nbc.gov.bt/portalBhutan
Species: Berberis insignis	Species Diversity of Bhutan, National Biodiversity
	Center, Bhutan (http://portal.nbc.gov.bt/portalBhutan
Species: Berberis macrosepala	Species Diversity of Bhutan, National Biodiversity
	Center, Bhutan (http://portal.nbc.gov.bt/portalBhutan
Species: Berberis praecipua	Species Diversity of Bhutan, National Biodiversity
	Center, Bhutan (http://portal.nbc.gov.bt/portalBhutan
Species: Berberis thomsoniana	Species Diversity of Bhutan, National Biodiversity
	Center, Bhutan (http://portal.nbc.gov.bt/portalBhutan
Species: Berberis tsarica	Species Diversity of Bhutan, National Biodiversity
	Center, Bhutan (http://portal.nbc.gov.bt/portalBhutan
Species: Berberis virescens	Species Diversity of Bhutan, National Biodiversity
	Center, Bhutan (http://portal.nbc.gov.bt/portalBhutan

Table 2. Berberis Spp. reported to occur in Bhutan (source: National Biodiversity Centre, Bhutan)

21/04/2012 27.23542 89.4869 21/04/2012 27.25663 89.50958 21/04/2012 27.27737 89.51443
89.4869 2413 Susuna, Dogor, F 89.50958 2597 Sali, Dogor, Paro 89.51443 2494 Sali, Dogor, Paro
2413 Susuna,Dogor,Paro 2597 Sali,Dogor,Paro
field
null
l none

Table 3. Survey sites data summary

none	none	none n	flower	Barley	field	Naja	2584	89.48902	27.2038	21/04/2012
none none		-	flower	Barley	field	Dhoakhar	2570	89.50988	27.25683	21/04/2012
none none		_	flower	Barley	field	Naja, rochika	2652	89.44089	27.22704	21/04/2012
none none			boot	BW	field	Dhogacar	2463	89.51398	27.27745	21/04/2012
none Low			boot	BW	field	Naja	2362	89.483	27.23439	21/04/2012
none none			boot	BW	field	Susuna,Dogor,Paro	2431	89.48735	27.23102	21/04/2012
none none	_		boot	barley	field	Labana,Naja,Paro	2622	89.48948	27.2037	21/04/2012
none none			flower	barley	field	Naja, Paro	2683	89.44054	27.2272	21/04/2012

BW = Bread Wheat

Incidence and Severity Scores: Low = 1-20%, Mod = 20-40%, High (>40%)



Photo 1. Stripe Rust Infected Wheat Crop, Punakha Valley 17th April 2012

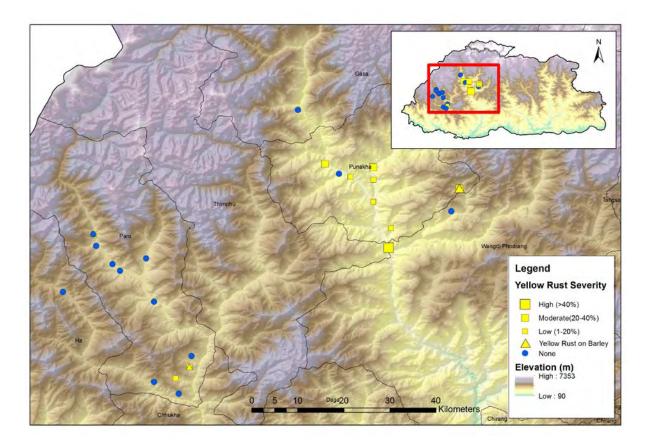
Photo 2: Example s of Berberis sp. observed on the survey



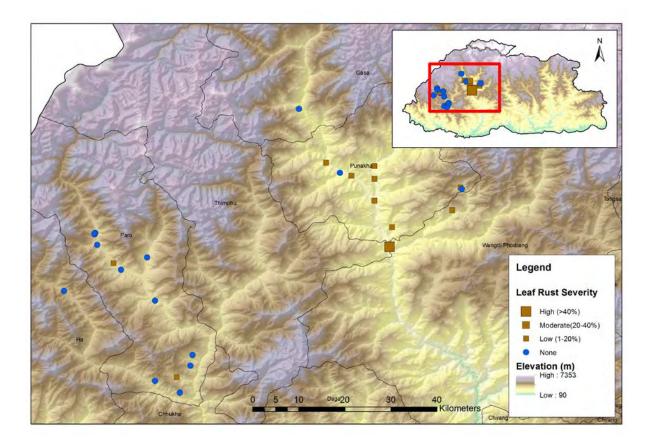
Photo 3: Berberis Sp. with aecial infections



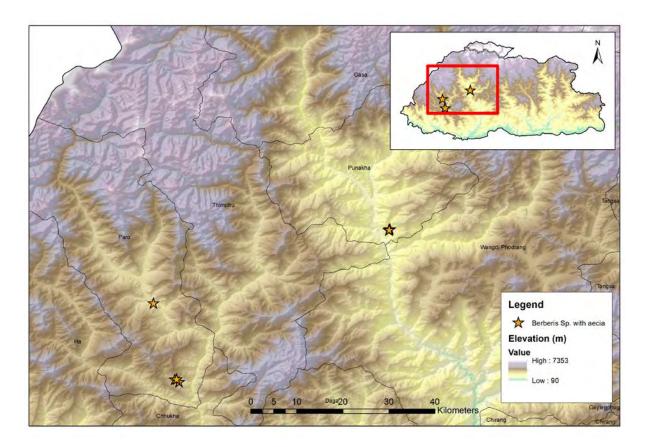
Map 1: Survey Sites - Yellow Rust Severity

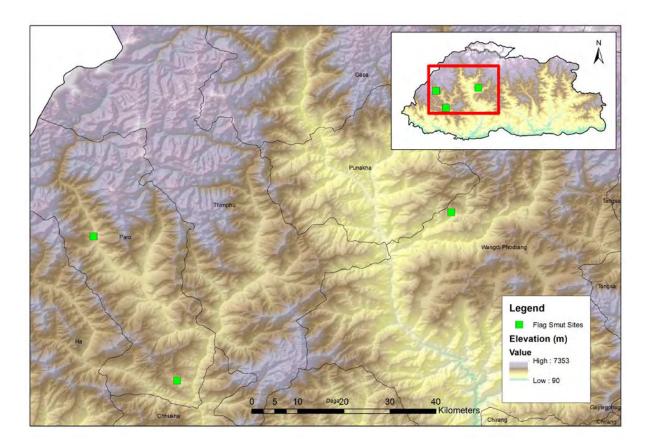


Map 2: Survey Sites - Leaf Rust Severity



Map 3. Locations of Berberis Sp. Infected with Aecia





Map 4: Survey sites infected with Flag Smut (Urocystis agropyri)