The Potential of Rust as a Biological Control of Parthenium in Ethiopia

Taye Tessema[1], G Einhorn[2], M Gossmann[2], C Büttner[2] and R Metz[2]
[1]*Plant Protection Research Centre, Ethiopian Agricultural Research Orgnaization, P.O. Box 37, Ambo, Ethiopia*
[2]*Faculty of Agriculture, Humboldt University of Berlin, Lentzealee 55/57 14195 Berlin, Germany*

Abstract

Parthenium is an invasive annual weed that is widely spread in Ethiopia. Parthenium rust caused by the rust fungus *Puccinia abrupta* var. *partheniicola*, has been used as a biological of the weed. The rust was found to be effective and was introduced to Australia from Mexico as bio-control agent. The current study was done to determine the rust's incidence and distribution, virulence and host-specificity, and its effects on the morphological and seed production capacity of parthenium during 1998–2002. The rust was found in mid-altitude (1500–2400 m) regions of Ethiopia with over 50% incidence. As a result of rust infection, reductions were observed in mean plant height was 11.1%, number of leaves per plant 21.5%, leaf area 27.5%, number of branches 13.4%, dry matter yield at maturity 24.8% and number of seeds produced 42.7%. Comparison of *P. abrupta* isolates showed that Ambo and Debre Zeit isolates were comparatively the most virulent based on the number of pustules per leaf and number of leaves infected. Host specificity study on weed and related crop plants revealed no apparent infection except on parthenium. Microscopic examination using leaf clearing and staining technique showed host reaction with poor development of uredospores on *Guizotia abyssinica* varieties. The rust shows significant potential as a classical biological control of parthenium in mid altitude areas of Ethiopia.

Key words: Parthenium, rust, biological control

Introduction

Parthenium (*Parthenium hysterophorus*) is the major invasive weed both in arable and grazing lands in Ethiopia. It is an annual herb that germinates any time of the year and grows rapidly to a height of 0.5–2 m. It is classified under the family Compositae, tribe Helianthae and sub-tribe Ambrosiinae. The major ecological and morphological characteristics that contribute to its severe invasiveness might be its adaptability to wide climatic and soil conditions, production of allellochemicals and ability to produce large number of seeds (10.000–25.000 per plant) (Navie et al. 1996). The seeds, which are small (1–2 mm diameter) and light (50 μg), are able to travel long distances through wind, water and other means. A yield reduction of 40% in agricultural crops and 90% in forage production in grasslands were reported (Khosla and Sobti 1979, Nath 1998). In eastern Ethiopia, parthenium is the second most frequent weed (54%) after *Digitaria abyssinica* (63%) in sorghum reducing grain yield from 40 to 97% depending on the year and the location (Tamado 2001). In addition to direct competition for resources and allelopathic effects to its crop host, it causes health hazard to humans and animals (Chippendale and Panneta 1994, Kololgi et al. 1997). The use of pathogens and insects as biological control agents is recommended as an element of integrated parthenium management by the Parthenium Action Group (PAG 2000). The rust fungus, *Puccinia abrupta* var. *partheniicola*, was introduced into Australia in 1991 from Mexico as a classical biological control of parthenium. The fungus is an autoecious, macrocyclic that has a severe impact on its host causing reduction in vegetative growth and seed production (Evans 1987). Studies made by Parker et al. (1994) indicated that the pathogen was host specific and completed its lifecycle only on *P. hysterophorus* and the closely related *P. conferatum* though minor symptoms such as chlorosis and necrosis were observed without any sporulation on some sunflower cultivars. Taye (2002) suggested that the rust was introduced to Ethiopia possibly together with its host from Kenya and/or Somalia since its earlier presence in Kenya, in 1977, was reported (Evans 1977).

The objectives of the present study were to determine the incidence and distribution of *P. abrupta* in the northern, central and eastern parts of Ethiopia; assess its effect on parthenium under field conditions; and test its pathogenecity on major economic crop and weed species related to parthenium in Ethiopia.

Materials and Methods

Incidence and distribution of *Puccinia abrupta*

The incidence and distribution of *P. abrupta* were recorded in major parthenium infested areas of Ethiopia: the central and Rift Valley, South and North Wollo, West and East Harerge, and East Wollega during 1999–2002 (Figure 1). The incidence of the disease was assessed in cultivated lands, fallow lands and grasslands. The assessment was done as per cent of parthenium plants with a rust symptom over the total plants in 4 m x 4 m plots (16 m^2). Five counts were taken per field and 3–5 fields were assessed at random at an interval of 2–3 km per location and then scaled as < 1%, 1–5%, 6–20%, 21–50% and > 50% (PPRC 2000).

Pathogenicity test on parthenium

Rust spores were harvested from diseased plant material from Ambo, Debre Zeit, Asebe Teferi, Alemaya and Hirna since these areas were known to be parthenium infested and with a relatively high incidence of the rust. The spores were scratched off from air-dried leaves and stems and inoculated to parthenium plants in the greenhouse for maintenance. Three-week-old uredospores of each isolate were collected from fresh plants in greenhouse and used for inoculation (Parker et al. 1994). The spores were gently shaken and mixed with a sterile solution of 0.01% Tween 80 in distilled water. Five isolates were tested for their viability/germination by carrying out spore germination test by germinating spores on special slides under moist condition. Concentration of approximately 10^5 spores per ml was prepared for each isolate. The suspensions were applied both onto the lower and the upper leaf surfaces of parthenium with a soft-hair brush for maximum coverage. All leaves were inoculated at 4–6 leaf stages. To establish the infection, plants were sprayed fine droplets of water to create dew, and then put in a plastic chamber to maintain a maximum. Inoculated plants were maintained at about 17 °C for about 24 hr and then placed in the greenhouse and wirehouse conditions. Data on the number of infected leaves and number of pustules developed on the upper and lower leaf surfaces were recorded and later subjected to analysis of variance using MSTATC software package.

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Spore germination test

Special glass slides with 10 slots were used for spore germination test. Uredospore suspension of each isolate at spore concentration of 10^5 per ml was prepared in 10-ml plastic test tube containing 0.01% Tween 20 solution. A 20-µl suspension of each isolate was placed into each slot of the glass slide in replicate. Each glass slide was then placed on wet filter paper in petriplate and then covered with aluminium foil. The plates were incubated at 17 and 20 °C for 6 to 10 hr.

Host specificity of *Puccinia abrupta* on some related species

The selection of test plants was based on the phylogenetic centrifugal testing sequence of Wapshere (1974) and similarities of plant species tested at International Institute of Biological Control (IBBC) by Parker et al. (1994) and Seier et al. (1997). Crop species used in host-range studies were three varieties of niger seed (*Guizotia abyssinica* L.) sesame (*Sesamum indicum* L.), safflower (*Carthamus tinctorius* L.), watermelon (*Citrullus vulgaris* Schrad.) and cucumber (*Cucumis sativus* L.). The niger varieties were Kvyu, Esette, and Fogera. The related weed species were *Xanthium strumarium*,. *Guizotia scabra, Tagetus minuta, Bidens pilosa, Sonchus* sp., *Bidens schimperii* and *Parthenium hysterophorus* as a control.

Seeds were sown in a plastic plate of 20 cm x 40 cm x 5 cm consisting 1:1:1 mixture of sand, humus and clay. The seedlings were then transplanted 2–3 weeks after sowing in 12 cm diameter pots. Two weeks after transplanting, plants having uniform growth were selected and then inoculated at 4–6 leaf stages with the uredospores of Ambo isolate based on its relatively high spore germination capacity as assessed during the initial phase of the study. Host infection was confirmed both by visual assessment of symptoms of pustule development and also by microscopic examination of inoculated tissues according to the technique of Bruzzese and Hasan (1983) and Parker et al. (1994).

Effects of *puccinia abrupta on* morphological parameters and seed production

Assessment of the effects was carried out by comparing the diseased and the healthy plant samples collected from two locations, namely, Ambo and Dukem. Two separate plant sample collections were made determine the effects on morphological parameters and seed production.

For the effects on morphological parameters, 50 rust-infected and rust free parthenium plants each were randomly collected at flowering stage by walking diagonally in maize field and grassland, respectively, both at Ambo and Dukem. The morphological parameters considered were plant height, number of leaves per plant, leaf length, leaf width, number of branches and dry matter weight. Data from the two locations were square root transformed and analysed using MSTATC software package. The least significant difference (LSD) test was used to separate the means.

The plants were placed in polyethylene sack and dried in glasshouse using sun. The floral parts were threshed and separated from the rest of the plant and weighed. A sample of 5 g. from the threshed seed part were taken for healthy versus infected samples, and spread onto a sheet of paper and number of seeds was counted using a hand lens.

Results

Occurrence and distribution of *Puccinia abrupta*

The cause of parthenium rust was identified as *P. abrupta* var. *partheniicola*

(Jackson) Parmelee (Parmelee, 1967). The pustules, uredospores and teliospore of the fungus are shown on Figure 2. The rust fungus was found infecting leaves, stems and floral parts of parthenium plants in cool and humid regions of the surveyed areas. Per cent incidence of *P. abrupta* under different habitat of parthenium infestation in Ethiopia is presented in Table 1. Incidence of the rust varied from 0 to 100% in different areas. The highest incidence (> 50%) was observed at Mersa area (North Wollo) and Kombolcha (town, roadsides and in harvested maize fields) in South Wollo in January 2000. In Woldiya, Addis Mender, Ambo, Hirna, and Qarsa, the rust incidence was 21–50% both on roadsides and crop fields after harvest. During the crop growing period of 2000, the rust incidence was very low (1–5%) in North and South Wollo. However, in central and eastern Ethiopia, incidence of greater than 50% was recorded in Debre Zeit, Asebe Teferi, Hirna, Kobo, Kersa, and Alemaya. While in Ambo, Akaki and Mojo areas, incidence of 21–50% was recorded. In relatively warm temperature areas like Gobiye, Koqa, Nazeret, Welenchiti, Harer and Feddis, incidence was very low (1–5%). In contrast the rust was not found in areas of hot and dry climate and at areas of low altitude like Kobo, Shewa Robit, Metehara, Awash and Dire Dawa.

Germination of *P. abrupta* spores

There was no significant difference in per cent spore germination between spores incubated at 17 °C and 20 °C (Table 2). Per cent spore germination at 17 °C after 6 and 10 hr of incubation were 18.5% and 21.5%, respectively. Whereas, at 20 °C, it was 18% and 23% after 6 and 10 h of incubation, respectively. This shows that per cent spore germination increased with increased time of incubation.

Comparison of virulence of *P. abrupta* isolates

Significant variation was observed among the *P. abrupta* isolates collected from different locations in mean number of infected leaves per plant and mean number of pustules per leaf on the upper and lower leaf surfaces at 14 and 21 days after inoculation both in the greenhouse and lath house (Tables 3). The highest number of leaves with upper surfaces attacked per plant was recorded from Ambo isolate although there was no significant difference between isolates. Similarly, there was no significant difference among the tested isolates based on the number of leaves with lower surfaces attacked except Alemaya isolate which produced the lowest at 14 days after inoculation. At 21 days after inoculation, however, Ambo isolates had significantly higher number of leaves with lower leaf surfaces attacked, while there was no significant difference among the other isolates. The highest mean number of pustules on the upper and lower leaf surface/leaf were also recorded from Ambo and Debre Zeit isolates both at 14 and 21 days after inoculation. Asebe Teferi, Hirna and Alemaya isolates showed similar virulence considering all parameters except on number of leaves with the upper leaf surface infected 14 days after inoculation at which Alemaya isolate showed the lowest (Table 3).

A similar trend was obtained in lathhouse when compared to greenhouse experiment. Ambo and Debre Zeit isolates resulted in higher number of leaves with upper and lower leaf surfaces attacked. The highest mean number of pustules was produced by Ambo isolate followed by that of Debre Zeit while Alemaya isolate was found to be the least both at 14 and 21 days after inoculation (Table 3). In general, Ambo isolate was found the most virulent followed by Debre Zeit isolate. Alemaya isolate, on the other hand, was found the least virulent both in greenhouse and lathhouse experiments.

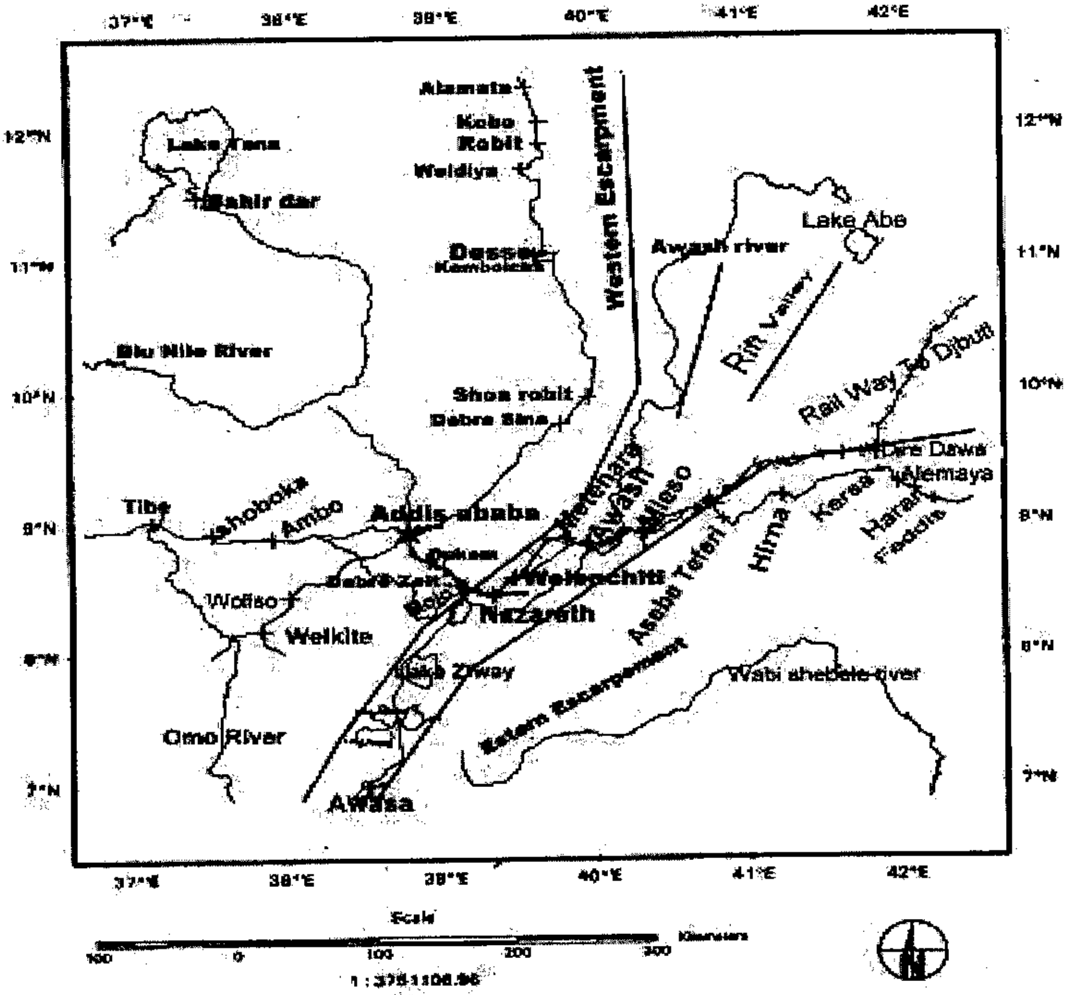

Figure 1. Distribution of parthenium rust in the northern, central and eastern parts of Ethiopia

Host specificity of *P. abrupta* on related crop and weed species

Sporulation *of P. abrupta* was observed only on parthenium. However, limited numbers of poorly developed pustules were observed on all 3 varieties of niger seed. Whereas no disease symptom was observed on all other crop and weed species tested. Hence, they were rated as immune or non-host. Mean number of leaves attacked per plant and mean number of poorly developed pustules per leaf three weeks after inoculation with *P. abrupta* were highest on niger seed variety Kuyu (Figure 3). This suggests that there exists variation in the degree of reaction among the three varieties of niger seed against *P. abrupta*.

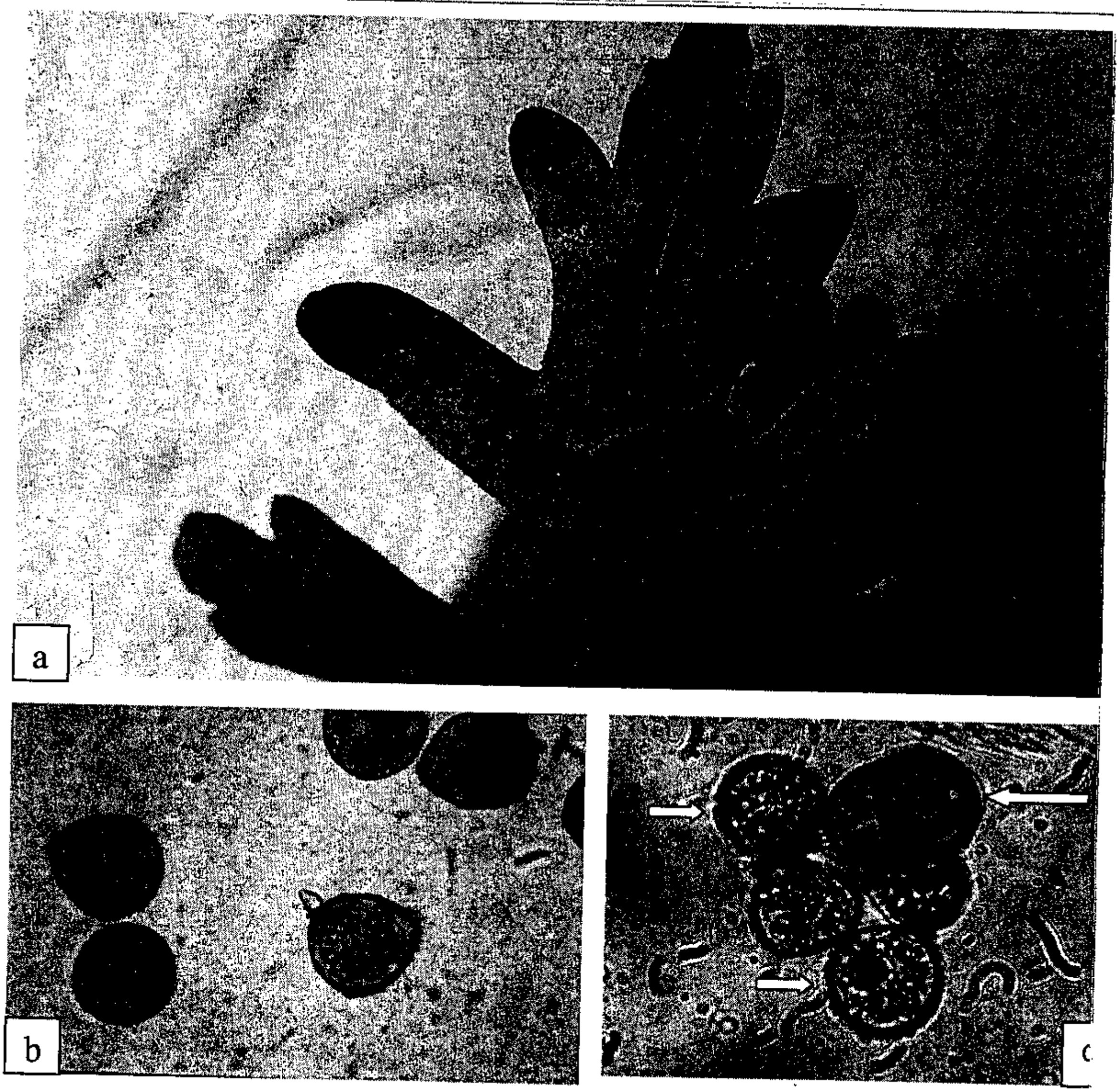

Figure 2. *Puccinia abrupta* var. *partheniicola*: a) pustules with uredospores produced 21 days after inoculation in the greenhouse; b) uredospores; c) teliospore (long arrow) and uredospores (short arrows)

Effects on morphological parameters and seed production

The effect of the rust on morphological parameters at flowering at Ambo and Dukem is shown in Table 4. Mean plant height, number of leaves per plant and number of branches per plant were higher for parthenium at Ambo than at Dukem although these parameters could be affected by various edaphic and environmental factors in addition to the rust in both locations. However, mean leaf length, mean leaf width and mean leaf areas were reduced by rust more at Ambo than at Debre Zeit (Table 4).

Similarly, dry matter yield at maturity and mean number of seeds per plant were higher at Ambo than at Dukem. Dry matter yield at maturity and mean number of seeds per plant were significantly reduced by 24% and 46%, respectively, as compared to healthy plants at Dukem (Figure 4). Similarly, 26% reduction in dry matter yield and 40% reduction in mean number of seeds per plant were obtained at Ambo.

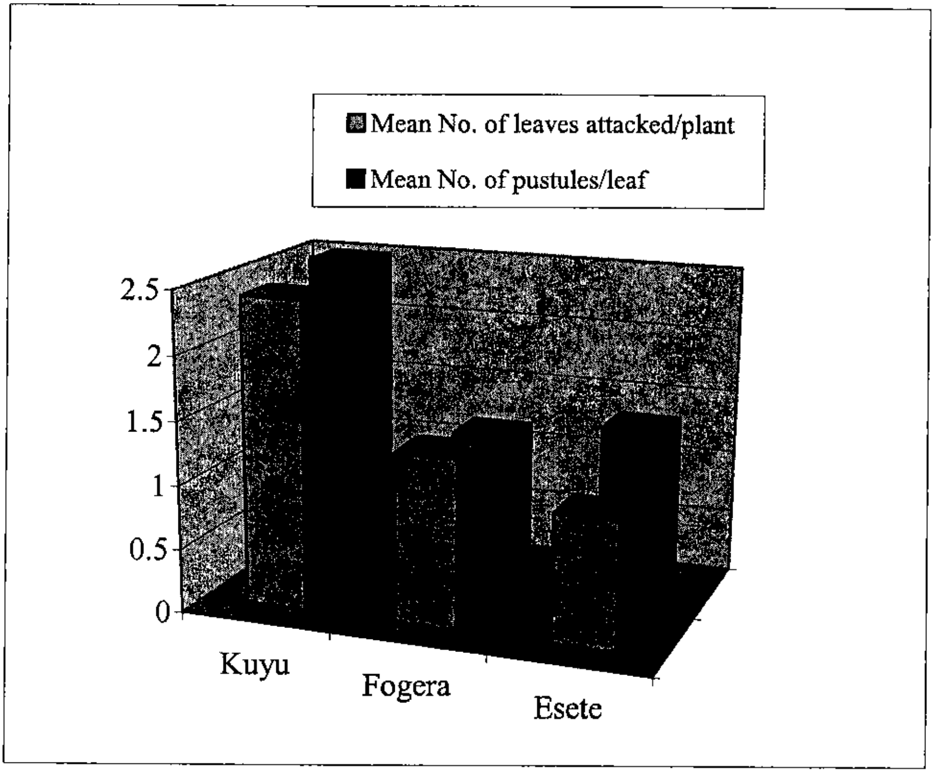

Figure 3. Mean number of leaves attacked per plant and mean number of poorly developed pustules per leaf due to *P. abrupta* on three varieties of niger seed (*Guizotia abyssinica*) (n = 36)

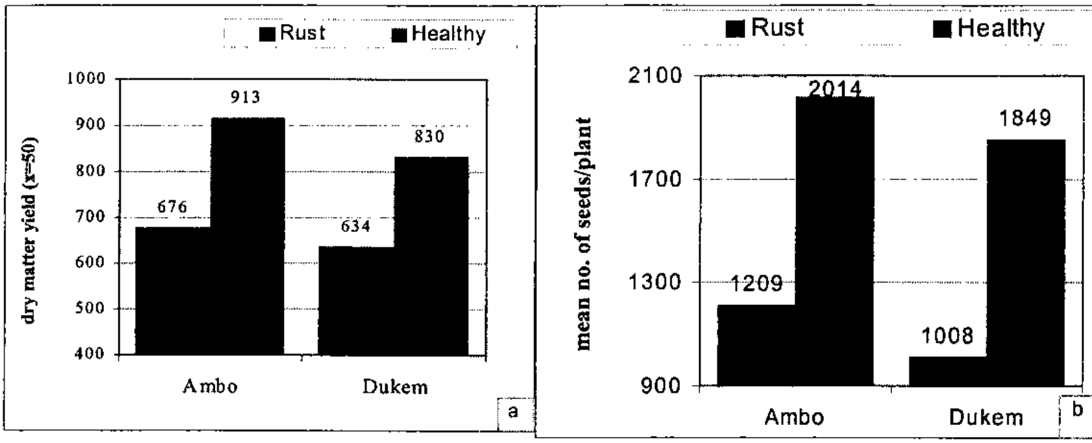

Figure 4. Dry matter yield (a) and number of seed produced per plant (b) by rust-infected and healthy parthenium plants at Ambo and Dukem

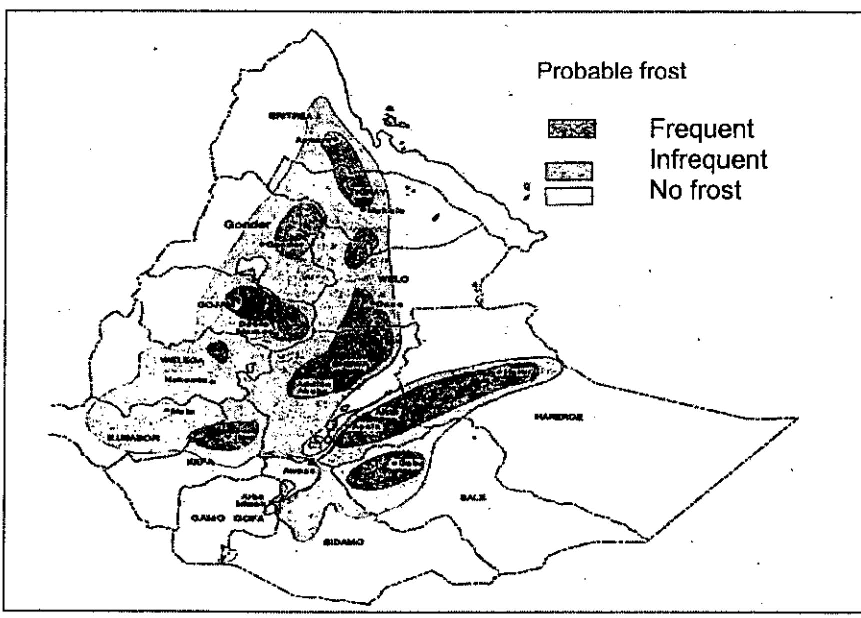

Figure 5. Probable frost occurrence in different parts of Ethiopia (EMA)

Table 1. Incidence and distribution of parthenium rust in northern, central and eastern parts of Ethiopia, 2000

Zone	Location	Altitude (m)	Habitat	Incidence %	
				Jan– Feb	Oct.– Nov
North Wollo	Kobo	1470	Sorghum	-[a]	-
	Robit	1500	Sorghum and maize	6 – 20	-
	Gobi	1580	Sorghum	1 – 5	-
	Woldiya	1880	Sorghum. Grassland	21 – 50	-
	Mersa	2300	Road side	> 50	-
South Wollo	Kombolcha	1903	Maize	> 50	1 - 5
	Addis Mender	1480	Maize	21 – 50	1 - 5
East Wollega	Sibusire	1750	Road side	1 – 5	*[b]
West Shewa	Shoboka	1750	Road side	1 – 5	*
	Ambo	2225	Maize	21 – 50	21 - 50
	Welliso	2000	Road side	*	1 - 5
	Tullubollo	2100	Road side	*	6 - 20
East Shewa	Akaki/Dukem	2050	Road side	-	21 - 50
	Debre Zeit	1900	Road side	6 – 20	> 50
	Mojo	1870	Maize. road side	1 – 5	21 - 50
	Qoqa	1600	Road side	*	1 - 5
	Nazeret	1620	Maize. road side	1 – 5	1 - 5
	Wolanchitti	1450	Maize. Tef	1 – 5	1 - 5
	Matahara	970	Maize. sorghum	-	-
Afar	Awash	920	Sorghum	-	-
	Anano	1200	Sorghum	-	-
	Miesso	1400	Sorghum	-	< 1
West Harerge	Asebe Teferii	1900	Maize. sorghum	6 – 20	> 50+
	Hirna	2050	Road side. grass land	21 – 50	> 50+
	Chelenko	2350	Road side	6 – 20	*
	Qobo	2250	Road side	-	> 50+
East Harerge	Qulubii	2000	Maize. sweet potato	*	*
	Qarsa	2000	Sorghum. road side	21 – 50	> 50+
	Dire Dawa	1360	Sorghum	-	-
	Tony Farm	1260	Fruit	-	-
	Alemaya	2125	Maize and sorghum	6 –20	> 50
	Harer	2100	Road side	1 – 5	-
	Feddis	1700	Vegetable	1 – 5	-

[a] = not observed

[b] = not assessed

Table 2. *Puccinia abrupta* spore germination after 6 and 10h of incubation at 20 and 17 °C using freshly harvested uredospores of ambo isolate

Parameter	After 6 h		After 10 h	
	20 °C (n = 200)	17 °C (n = 200)	20 °C (n = 200)	17 °C (n = 200)
No. of spores germinated	36	37	46	43
Per cent spore germination	18	18.5	23	21.5

Table 3. Virulence of *Puccinia abrupta* isolates collected from different locations of Ethiopia in the greenhouse

Isolate	Mean number of infected leaves /plant		Mean number of infected pustules /leaf	
	Upper surface	Lower surface	Upper surface	Lower surface
14 days after inoculation (n = 80) in greenhouse				
Ambo	4.0 a	2.9 a	16.4 ab	12.0 a
Debre Zeit	3.4 ab	2.7 a	19.8 a	12.4 a
Asebe Teferi	2.8 b	2.4 a	12.2 b	6.0 b
Alemaya	2.1 c	1.8 b	10.7 b	3.9 c
Hirna	2.9 b	2.3 a	12.8 b	6.8 b
21 days after inoculation (n = 40) in greenhouse				
Ambo	4.3 a	3.5 a	26.0 b	17.1 a
Debre Zeit	3.4 ab	3.0 ab	24.9 a	12.0 a
Asebe Teferi	2.9 b	2.5 b	13.9 b	7.0 b
Alemaya	2.8 b	2.6 ab	10.6 b	5.9 b
Hirna	3.0b	2.5 b	12.8 b	6.8 b
14 days after inoculation in wirehouse (n = 80)				
Ambo	3.1 a	3.1 a	27.7 a	8.3 a
Debre Zeit	3.0 a	2.6 a	21.6 ab	5.0 b
Asebe Teferi	2.9 a	1.9 b	15.8 bc	3.1 c
Alemaya	2.0 b	1.2 c	10.7 d	2.1 c
Hirna	2.5 a	1.4 bc	14.6 cd	3.4 bc
21 days after inoculation in wirehouse (n = 40)				
Ambo	3.5 a	3.1 a	26.2 a	9.2 a
Debre Zeit	3.6 a	2.7 ab	19.0 b	5.7 b
Asebe Teferi	3.1 ab	2.3 bc	15.9 b	5.1 bc
Alemaya	2.5 b	1.7 d	10.7 c	3.7 c
Hirna	2.6 b	1.8 cd	18.3 b	4.2 bc

Table 4. Effect of *Puccnia abrupta* on morphological parameters of partheniumweed during flowering at two locations

Parameters	Ambo			Dukem			Mean reductio n (%)
	Healthy (n = 50)	*P. abrupta* infected (n = 50)	Reducti on (%)	*P. abrupta* infected (n = 50)	Healthy (n = 50)	Reduction (%)	
Plant height (cm)	79.2	70.4	11.1	52.9	52.9	11.1	11.1
No. of leaves/plant	99.2	78.7	20.7	45.5	34.9	13.4	17.1
Mean leaf length (cm)	9.0	7.1	21.1	8.1	7.7	5.0	13.1
Mean leaf width (cm)	4.8	3.5	27.1	4.3	4.1	4.7	15.9
Mean leaf area (cm^2)	46.3	28.0	39.5	38.4	33.1	13.8	26.7
Mean no. of branches/plant	7.7	7.0	9.1	5.3	4.8	13.4	11.2

Discussion

P. abrupta var *partheniicola* was observed successfully infecting parthenium in many infested areas. The fungus caused significant reduction in seed production and plant development in the field. The rust was commonly found in cool mid-altitude (1500–2500 m) regions of the surveyed areas where rainfall ranged 700–1400 mm and the frost occurrence was frequent (Figure 5). This could be due to the requirement of low temperature and leaf wetness in the form of dew for germination of spores and subsequent development of infection. The spore germination test showed that germination started 5 hr after incubation at 20 °C and an increase in per cent germination occurred with an increase in time of incubation indicating that a dew period of at least 5 hr is required to initiate infection (data not shown). This is in agreement with the result of Parker et al. (1994) which indicated that low level of pustule formation were found with dew periods of 4–6 hr while it was higher from 7 hr and higher of leaf wetness in the greenhouse.

Rust incidence was low or did not exist in areas with altitudes of less than 1400 m which are generally characterized as warm. This might be due to high night temperatures resulting in absence of dew to stimulate spore germination. The disease incidence was invariable in different habitats such as on roadsides, grassland and crop field indicating its uniform spread. Similarly, Parker et al. (1994) reported that *P. abrupta* occurs only at elevated sites in Mexico (> 1000 m), Kenya (1490 m) and India (930 m) and that it was not recorded from Texas or Madagascar where parthenium occurs at low altitudes. Thus, *P. abrupta* can infect the weed in mid-cool altitude regions and can, therefore, be utilised in integrated parthenium management in these areas.

P. abrupta significantly reduced plant height, leaf number, leaf area, number of branches, dry matter yield and seed production. The reduction in leaf number and leaf area due to *P. abrupta* might reduce the amount of photosynthate produced, which in turn reduces plant height, number of branches that bear flowers and seed. Since parthenium reproduces by seed, rust infection curtails the seed production to a certain extent and consequently reduces the spread of the weed. In addition, rust-infected plants would be subjected to increased stress, and reduced competitiveness to the crop and other native plant species. This result also agrees with the study made by Parker et al. (1994) in the greenhouse through repeated inoculation of the rust. They found that infection with the rust increased leaf senescence, decreased the life span and dry weight, and reduced flower production ten fold. However, in their study, artificial inoculation failed to reduce plant height, leaf production or lateral branch formation which might be due to the early assessment of the disease (i.e., over four weeks in the middle of the growing period) as opposed to our field study that has been done during mid-flowering stage. This might also be due to the less severity of rust pustules developed on parthenium plant parts (leaves, stems, petioles and sepals) as opposed to the high intensity of rust pustules observed in the field. Hence, the assessment under natural field condition is more advantageous in showing the real effects of *P. abrupta* than assessment through artificial test in greenhouse.

Ambo and Debre Zeit isolates were relatively found to be the most virulent. This might be due to the influence of location on the virulence. A range of *P. abrupta* isolates, one from Kenya and five from Mexico, were collected and assessed in the United Kingdom for pathogenicity to the Australian parthenium plants based on pustule production (Parker et al. 1994). However, the authors indicated that no single isolate was consistently more virulent than any of others though Saltillo isolate produced the most vigorous infection at a high night temperature (> 20 °C).

In the host specificity test, all the tested crop and weed species were found to be resistant or immune to *P. abrupta*. Only *G. abyssinica* varieties showed limited number of poorly developed pustules. As opposed to this study, Parker et al. (1994) found no development of spores on *G. abyssinica* but only on *Parthenium conferatum* var. *lyratum* of the 120 species and varieties tested. They observed host responses such as necrosis around point of infection, cell wall granulation, callose formation around haustria or on host cell walls around infection or deposition of polyphenols around penetrated stomata in many of the test plants. Additional screening using different climatic and biotic parameters designed to stress or predispose the plants to rust infection also showed no further symptoms appeared and the rust was finally approved for biological control of parthenium by Australian Quarantine and Inspection Service (AQIS) and then released in 1991 (Parker et al. 1994).

In the current study, infection of *P. abrupta* on *G. abyssinica* was not observed under field condition. Conducting host range studies in artificial conditions might predispose plants to infection. Hence, the development of symptoms on *G. abyssinica* varieties through artificial inoculation in the greenhouse might arise due to increased disease pressure giving false positive results that would not arise in the field as reported by Evans (2000).

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