CRP: Livestock

Flagship: FP3 – Feeds and Forages

Cluster: Cluster 2

Activity: P1685 – Activity/Product Line 3.2.1: Improved feed & forage germplasm and new tools and technologies for breeding

Deliverable: D24591 - Report on methodology for screening of spider mites resistance in a diversity panel

Report on methodology for screening of spider mites resistance in a diversity panel

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Summary

Spider mites attack is the most important limitation for improved *Urochloa* (syn. *Brachiaria*) crops in Eastern Africa causing up to 100% loss during the dry seasons. Two spider mite species have been previously identified in Kenya attacking brachiaria grasses: *Tetranychus urticae* (Koch) and *Oligonychus trichardti* (Meyer). Variation in the response of brachiaria genotypes to *O. trichardti* attack was characterized among cultivars released by CIAT in Kenya. To identify potential parents for crosses as part of the breeding strategy for East Africa, it is needed to develop a high-throughput methodology for assessing antibiosis and tolerance in *Urochloa* spp. to spider mites.

An experimental unit for no-choice tests under greenhouse conditions was developed and tested. This was composed of a single-stem vegetative propagated plant caged in a transparent acetate cylinder with four openings covered with mesh. Mite survival percentage was recorded to assess antibiosis and damage was measured through color indexes based on green and yellow pixels of the shoot to assess tolerance. Populations of *T. urticae* reared in beans (*Phaseolus vulgare*) cv. Cerinza showed a strong antixenosis effect on Mulato II and Cayman cultivars with an avoidance behavior when fully infested bean leaves were placed on the hybrids. This behavior was observed also in laboratory and greenhouse no-choice tests. A predatory mite of the *Phytoseiulus* genera was collected by sampling cv. Toledo, Cayman, and interspecific hybrids fields at CIAT Cali, Colombia (3º29’54.45’’ N, 7º21’26.47’’ O). It is possible this prey are thrips as phytophagous mites were not found.

Objectives

1. Collection and colony establishment

Spider mites in *Urochloa* spp.

The main limitation for improved brachiaria grasses productivity in Africa are spider mites (Cheruiyot et al., 2020). Two species have been identified hitherto in *Urochloa* spp. crops in Kenya: *Tetranychus urticae* Koch (Acari: Tetranychidae) and *Oligonychus trichardti* Meyer (Acari: Tetranychidae) (Cheruiyot et al., 2018; Mutisya et al., 2018). In contrast, few reports have been found of mites attacking this crop in America. In Cuba, de la Torre et al. (2005) identified *Steneotarsonemus furcatus* (Acari: Tarsonemidae) in *Brachiaria plantaginea* L., and in Brazil *Catarhinus granatus* (Acari: Eriophyoidea) and *Eotetranychus herbicolus* (Acari: Tetranychidae) were described in *Brachiaria ruziziensis* by (Flechtmann, 2004).

Samplings were conducted at the interspecific breeding program testcross fields and at cv. Toledo, Cayman and Mulato II silvopastoral essays at CIAT Cali (3º29'54.45'' N, 7º21'26.47'' O). To assess the presence of mites in *Urochloa*, samples of shoots collected in various points at the sites were taken to the laboratory and checked under the stereoscope. Only mites of the genera *Phytoseiulus* were collected in all the fields (Fig. 1), probably feeding on thrips or phytophagous mites present in weeds.

Figure 1. Mite of genera *Phytoseiulus* found in *Urochloa* interspecific A. Mulato II and B. cv. Cayman.

***Tetranychus urticae* colony**

A *T. urticae* colony was established and maintained in *Phaseolus vulgaris* cv. Cerinza to have a permanent source of individuals for the host resistance tests (Fig 2).

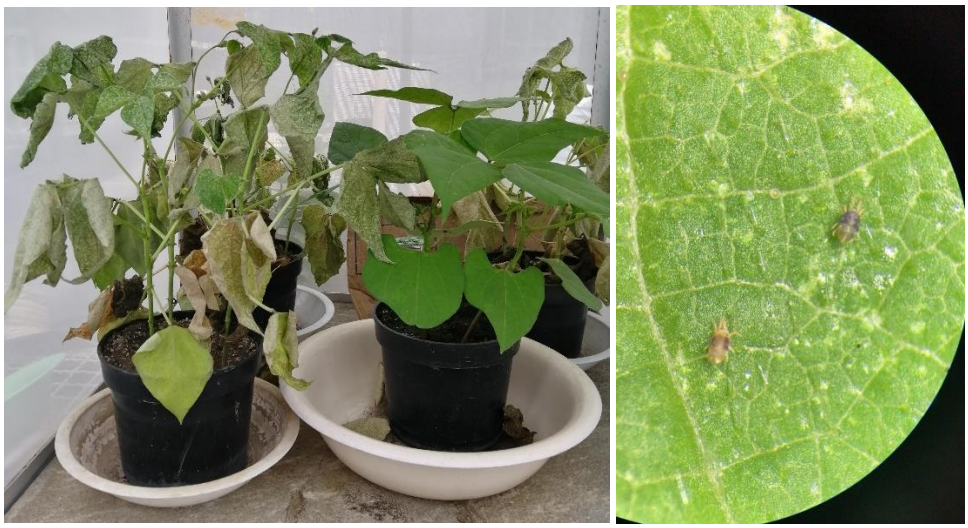

Figure 2. A. *Tetranychus urticae* colony on bean cv. Cerinza. B. Damage on bean leaf and oviposition.

To test the suitability of grasses, 10 fully infested bean leaves were placed in the shoot of five *Urochloa* interspecific cv. Mulato II plants placed in cages under environmental conditions at CIAT Cali entomology greenhouse (Fig. 3A). For the first two days, mites started to colonize the plants showing a walking behavior (Fig. 4A) through the leaves and stem. At the third and fourth day, they aggregated in the apex of the leaves to form silk balls full of individuals (Fig. 4B). This dispersal behavior is characteristic of eriophyoid and tetranychid mites when plants become overcrowded, the food is scarce, or the host plant is unsuitable (Clotuche et al., 2011; Kiedrowicz et al., 2017; Skoracka et al., 2007).

In a second essay, 15 fully infested leaves were placed on the leaves of one bean cv. Cerinza and two Mulato II plants placed in cages (Fig. 3B). Mites totally colonized the bean plants and occasionally walked on Mulato II plants. After the bean wilted, mites showed the same dispersal behavior on bean and Mulato II leaves (Fig. 5), and at the ninth day no live individual were found on the plants. This result suggests that cv. Mulato II is not a suitable host for this *T. urticae* Colombian population reared in beans, even though this cultivar was reported to be susceptible to *Oligonychus trichardti* and *T. urticae* and had shown an avoidance behavior. Our main hypothesis is that the Kenyan and Colombian populations diverge. A previous study reported that Kenyan *T. urticae* collected in brachiaria grass cultivars showed genetic differences with *T. urticae* from France, Spain, Mexico and Brazil (Mutisya et al., 2018).

Figure 3. Fully infested bean leaves on A. *Urochloa* interspecific cv. Mulato II leaves. B. on *Urochloa* interspecific cv. Mulato II and bean cv. Cerinza leaves.

Figure 4. A. Walking, B. Aggregation, and silk balls of *Tetranychus urticae* in *Urochloa* interspecific cv. Mulato II.

Figure 5. Aggregation and silk balls of *Tetranychus urticae* in A. beans cv. Cerinza and B. *Urochloa* interspecific cv. Mulato II.

2. Develop a methodology to assess antibiosis and tolerance to *Tetranychus urticae* Koch (Acari: Tetranychidae) in *Urochloa* spp.

Methodology for host-plant resistance tests

Characterization of antibiotic, antixenotic and/or tolerant genotypes among CIAT's gene bank collection, will allow the identification of potential resistance sources useful for the tropical forages breeding program. A reliable methodology to assess and categorize plant response to arthropod attack are no-choice tests. These tests ensure an even distribution of the insects on all the genotypes preventing the escape of the plants to the damage, however, it is more accurate to determine antibiosis and tolerance (M. Smith et al., 1994).

A panel of diversity of *Urochloa* spp. was sown and maintained under greenhouse conditions to be tested for resistance to *Tetranychus urticae*. Genotypes were selected under different criteria (Table 1). Firstly, cultivars already commercialized in Easter Africa and assessed in a previous study by (Cheruiyot et al., 2018). Secondly, a set of tetraploid accessions from the CIAT's gene bank. Lastly, a group of precommercial hybrids provided by Semillas Papalotla. Each genotype has four replicates (Fig. 6).

Figure 6. Panel of diversity of *Urochloa* spp. to be assessed for antibiosis and tolerance to spider mites.

Table 1. Genotypes of *Urochloa* spp. selected for assessment.

Accession name	Species
CIAT 606	*U. decumbens* cv. Basilisk
CIAT BR02/1752	*U. ruziziensis x U. decumbens x U. brizantha* cv. Cayman
CIAT 6294	*U. brizantha* cv. Marandu
CIAT 16125	*U. brizantha* cv. Piata
CIAT 26110	*U. brizantha* cv. Toledo (syn. Xaraes)
CIAT 36087	*U. ruziziensis x U. decumbens x U. brizantha* cv. Mulato II
CIAT 36061	*U. ruziziensis x U. decumbens x U. brizantha* cv. Mulato
CIAT 16107	*U. brizantha*
CIAT 664	*U. decumbens*
CIAT 6370	*U. decumbens*
CIAT 6426	*U. brizantha*
CIAT 6702	*U. decumbens*
CIAT 6735	*U. brizantha*
CIAT 16122	*U. brizantha*
CIAT 16348	*U. brizantha*
CIAT 26133	*U. brizantha*
CIAT 26183	*U. decumbens*
CIAT 26646	*U. brizantha*
CIAT BR02/1794	*U. ruziziensis x U. decumbens x U. brizantha*
CIAT BR02/0465	*U. ruziziensis x U. decumbens x U. brizantha*
CIAT BR04/3025	*U. ruziziensis x U. decumbens x U. brizantha*
CIAT BR04/3207	*U. ruziziensis x U. decumbens x U. brizantha*
CIAT BR06/0423	*U. ruziziensis x U. decumbens x U. brizantha*
CIAT BR05/1435	*U. ruziziensis x U. decumbens x U. brizantha*
CIAT BR05/1467	*U. ruziziensis x U. decumbens x U. brizantha*
CIAT BR09/3660	*U. ruziziensis x U. decumbens x U. brizantha*
CIAT BR09/4467	*U. ruziziensis x U. decumbens x U. brizantha*

i. Leaf disks tests

For spider mites, leaf disks assays are commonly used for measuring preference and resistance under laboratory or greenhouse conditions (Adesanya et al., 2019; Al-bayati, 2019; Cerna et al., 2009; de Resende et al., 2020; M. Smith et al., 1994). This methodology was adapted to perform no-choice tests with bean cv. Cerinza in controlled conditions (28ºC, 70% RH, 12h photoperiod). Pilot tests were performed with leaf disks (1.5cm) placed with the abaxial face up in petri dishes containing agar (5g plant agar L^{-1}) and infested with 30 adult females of *T. urticae* from the bean colony (Fig. 7), then survivorship and oviposition was measured counting live adults. The leaf disk was the experimental unit. On the next day, great damage and oviposition rate were observed, however, many individuals were dead showing walking behavior on the agar. This result suggests a poor availability of space and feed.

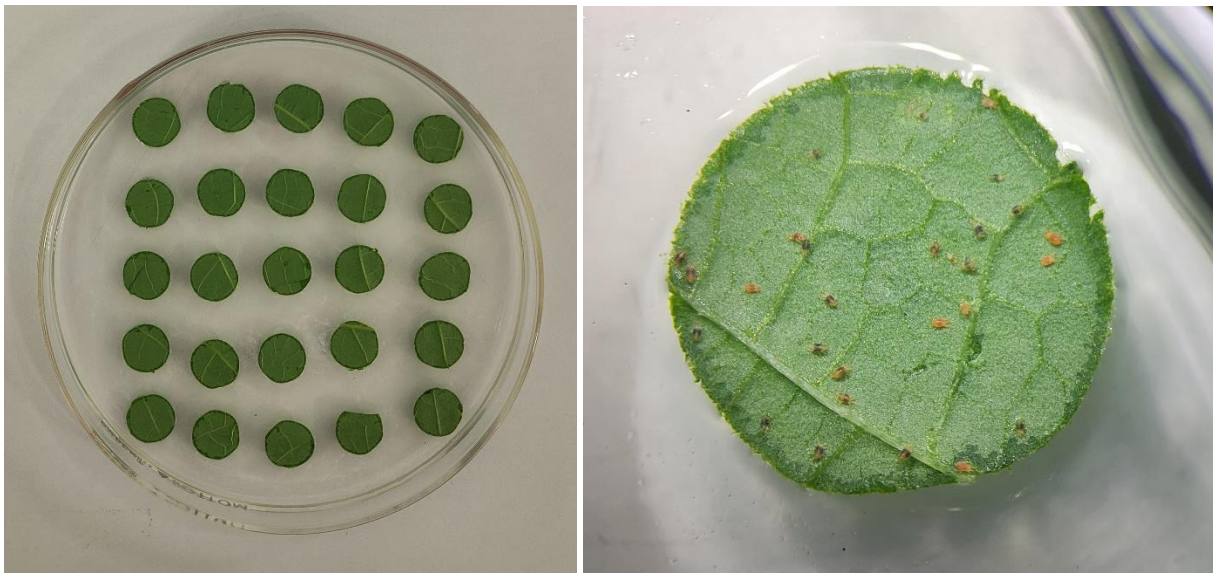

Figure 7. Leaf disk pilot test on bean cv. Cerinza.

A second pilot test was performed this time under greenhouse conditions and assessing brachiaria grass cv. Mulato II and bean cv. Cerinza. Leaf disks (1.5 cm diameter or length) were infested with 5 adult females. Survivorship and oviposition were assessed two days after counting live adults. On the bean leaves, females oviposited and fed in all replicates, however, in Mulato II, females showed an avoidance behavior (Fig. 8). In addition, eggs were found in two replicates of Mulato II (Fig. 9), but females were not feeding and showed a walking behavior on the agar.

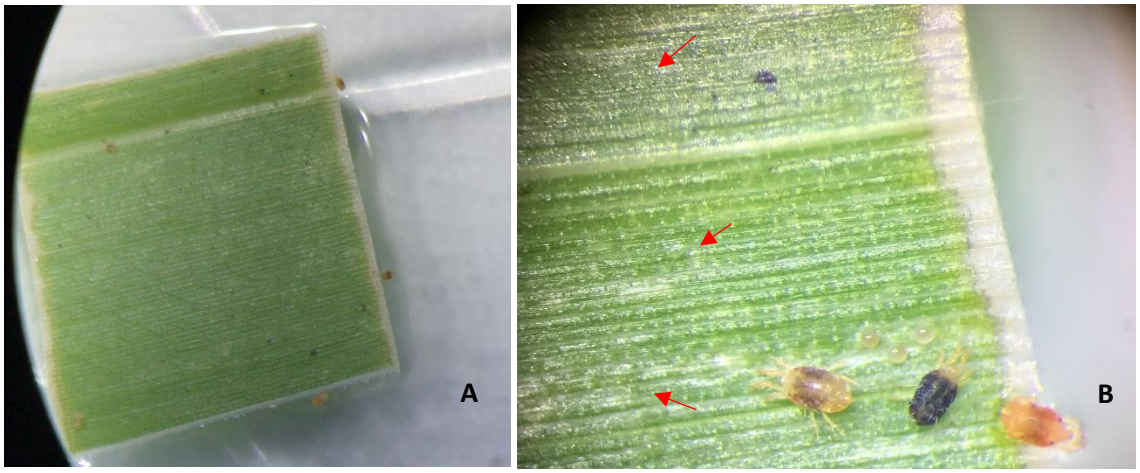

Figure 8. A. Avoidance behavior of *Tetranychus* urticae on leaf disk test on brachiaria cv. Mulato II. B. Oviposition on leaf disk test on *Urochloa* interspecific cv. Mulato II.

ii. *Seedlings greenhouse test*

Tests under greenhouse conditions allow to perform large scale evaluations on shoot feeders in a short period of time (Smith, 2005). Based on the methodology for screening large populations of *Urochloa* grasses to spittlebug (Hemiptera: Cercopidae) (Cardona et al., 1999) and barley to *Aceria tosichella* (Acari: Eriophyidae) (Aguirre-Rojas et al., 2019), it was designed an experimental unit of a single stem caged in a tube of acetate with three holes covered with veil.

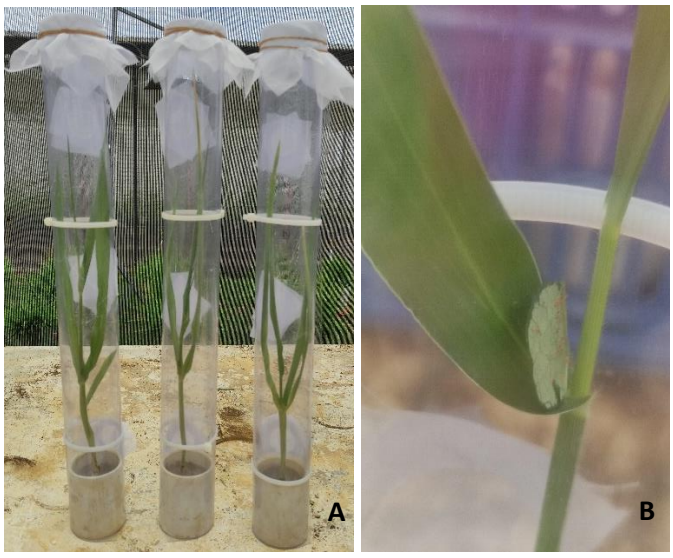

Figure 9. A. Brachiaria cv. Mulato II experimental units. B. Infestation of plants with *Tetranychus urticae*

Expected outcomes (next steps)

1. Test additional genotypes of *Urochloa* spp. to Kenyan spider mites attack in no-choice tests with leaf disk and seedlings methodology.
2. Assess damage caused by *Tetranychus urticae* through digital images analysis in beans.
3. Identify potential sources of resistance to *Tetranychus urticae* among the CIAT gene bank *Urochloa* spp. collection of tropical forages.

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