SESSION 1: TARGET AND AGENT SELECTION

Oral presentation

BIOLGICAL CONTROL OF PRICKLY ACACIA (VACHELLIA NILOTICA SUBSP. INDICA) IN AUSTRALIA:
NEW GALL-INDUCING AGENTS FROM AFRICA

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Abstract

Biological control is the most economically viable management option for prickly acacia (Vachellia nilotica subsp. indica), a serious weed of grazing areas in western Queensland, Australia. Biological control efforts so far have focused on agents from Pakistan, Kenya, South Africa and India, with limited success to date. Hence, the search for new agents, focusing on gall-inducers, was redirected to Ethiopia and Senegal, based on plant genotype and climate matching. Surveys were conducted on V. nilotica subspecies with moniliform fruits including the invasive subspecies indica. Prospective biological control agents have been identified based on damage potential, field host range and climate match. A gall thrips (Acaciothrips ebneri [Karny]) inducing shoot-tip rosette galls, a gall mite (Aceria sp. 3) deforming leaflets, rachides and shoot-tips in Ethiopia and Senegal and a tephritid fly (Notomma mutilum [Bezz]) inducing stem-galls in Senegal have been prioritized for further studies. The gall thrips from Ethiopia has been imported into quarantine in Brisbane, Australia and host specificity tests are in progress. The eriophyid gall mite from Ethiopia has been imported into quarantine in Pretoria, South Africa and host specificity tests are also in progress there. Results to date suggest that both agents are highly host specific at the subspecies level of the target weed. Future research will focus on the host specificity testing of the tephritid gall fly from Senegal which has been imported in quarantine in Brisbane, Australia.

Keywords: Acacia nilotica, gall thrips, Eriophyidae gall mites, Tephritidae gall fly, Ethiopia, Senegal

Introduction

Prickly acacia, Vachellia nilotica subsp. indica (Benth.) Kyal. & Boatwr. (syn: Acacia nilotica subsp. indica), a Weed of National Significance, is a serious weed of grazing areas in western Queensland and has the potential to spread throughout northern Australia (Dhileepan, 2009). Prickly acacia infests over 6 million hectares of natural grasslands and over 2,000 km of bore drains in Queensland, and costs primary producers AUD 9 million annually in lost pasture production (Dhileepan, 2009). Biological
control is the most economically viable management option for prickly acacia.

Biological control of prickly acacia in Australia commenced in the early 1980s, with native range surveys conducted in Pakistan, Kenya, South Africa and India (Dhileepan, 2009; Dhileepan et al., 2014). These surveys resulted in the introduction and establishment of only two agents to date, the seed-feeding bruchid Bruchidius sahlergi Schilsky from Pakistan and the leaf-feeding geometrid Chiasemia assimilis (Warren) from Kenya and South Africa. The impact of B. sahlergi on prickly acacia has been insignificant (Radford et al., 2001) while C. assimilis has established only at coastal sites, and not widely in the arid inland regions where the major infestations occur (Palmer et al., 2007).

Surveys in India identified five insects and two rust fungi as prospective biocontrol agents (Dhileepan et al., 2013). However, agents from India tested to date are either not sufficiently host specific or are proving difficult to rear in quarantine (e.g., Dhileepan et al., 2014; Taylor and Dhileepan, 2018). There are no other prospective agents identified in India.

A literature search (Dwivedi, 1993) and herbarium records suggested that prickly acacia (subsp. indica) and other V. nilotica subspecies with moniliform fruits occur in Ethiopia and Senegal. A CLIMEX model has also predicted that areas in Ethiopia and Senegal are climatically similar to the arid inland regions of western Queensland where prickly acacia is a major problem (Senaratne et al., 2006; Dhileepan et al., 2018). Hence, the search for new biological control agents were redirected to Ethiopia and Senegal. In this study, based on field host range, geographic range and damage potential, a gall thrips, a gall mite and a tephritid gall fly have been identified as additional prospective biological control agents for prickly acacia in Australia.

Materials and methods

Prickly acacia

The multipurpose tree Vachellia nilotica (L.) P.J.H. Hurter & Mabb. (syn: Acacia nilotica), native to Africa, the Middle East and the Indian subcontinent, is a polytypic species with nine recognized subspecies (Dwivedi, 1993). In Ethiopia, three V. nilotica subspecies, subsp. indica (Benth.) Kyal. & Boatwr. in the east (Afar and Oromiya regions), subsp. tomentosa (Benth.) Kyal. & Boatwr. in the north (Amhara region), and subsp. leiocarpa (Benth) Kyal. & Boatwr. in the south (Arbaminch region) were found growing naturally (Figure 1). In Senegal, two V. nilotica subspecies, subsp. tomentosa along the Senegal River and subsp. adstringens (Schumach. & Thonn.) Kyal. & Boatwr. in drier inland areas were found growing naturally (Figure 2). The subsp. indica and subsp. tomentosa have moniliform fruit pods (necklace-like, narrowly constricted between the seeds); and the subsp. adstringens and subsp. leiocarpa have non-moniliform fruit pods (not necklace shaped) with their margins straight or crenate or irregularly constricted (Dwivedi, 1993). The invasive prickly acacia in Australia (subsp. indica) is similar to subsp. tomentosa, but in subsp. tomentosa young branchlets and fruits are densely white-tomentose, while in subsp. indica young branchlets and fruits are glabrous to sub-glabrous or thinly pubescent (Dwivedi, 1993).

Native range survey for potential agents

In Ethiopia, surveys were conducted in July 2014 (22 sites), December 2015 (41 sites), November 2016 (10 sites) and November 2017 (12 sites) (Figure 1). In Senegal, surveys were conducted in April 2017 (8 sites), October 2017 (18 sites) and April 2018 (12 sites) (Figure 2). In both countries, the subspecies status of the prickly acacia population was recorded and insects and mites associated with various subspecies were collected. A greater emphasis was placed on gall-inducing agents, in view of their likely high host specificity. When arthropods were collected from prickly acacia, related co-occurring Fabaceae species were also checked at the survey sites, specifically Vachellia seyal (Delile) P.J.H.Hurter, V. tortilis (Forssk.) Galasso & Banč., V. ethaba (Schweinf.) Kyal. & Boatwr., V. abyssinica (Hochst. ex. Benth.) Kyal. & Boatwr., V. lahai (Steu. & Hochst. ex Benth) and Senegalia senegal (L.) Britton trees. The insects and mites collected were sent to relevant taxonomic experts in South Africa, Turkey and Australia for identification.
Host specificity tests

A subset of the test plants used in the host specificity testing of previous prickly acacia biological control agents from India (Taylor and Dhileepan, 2018) were used for testing the agents in quarantine in Australia and South Africa. The gall thrips and the gall mites from Shewa Robbit, Ethiopia were exported to a quarantine facility at Plant Health and Protection (PHP), Pretoria, South Africa in July 2014 and December 2017, respectively. Based on preliminary host specificity tests in quarantine in Pretoria, South Africa, the gall thrips were imported from Ethiopia into a high security quarantine facility in Brisbane, Australia in December 2015 for detailed host specificity tests.

Results

Gall thrips

In Ethiopia, the gall thrips Acaciothrips ebneri (Karny) (Thysanoptera: Phlaeothripidae) induced rosette galls in the axillary and terminal buds resulting in shoot-tip dieback (Figure 3a). The gall thrips were found only on subsp. tomentosa (74% of sites, n = 22) and subsp. indica (92% of sites, n = 24) and not on subsp. leioarpa (n = 8 sites). There was no evidence of the gall thrips on co-occurring V. abyssinica and V. eibatca trees in Ethiopia. In Senegal, the gall thrips (Figure 3b) was seen on both subsp. tomentosa (100% of sites, n = 13) and subsp. adstringens (88% of sites, n = 7), and not on co-occurring V. seyal, V. tortilis and ś. senegal trees.

In no-choice tests in the quarantine facility at PHP, South Africa, adult thrips from Ethiopia induced galls only on Australian prickly acacia (subsp. indica with moniliform fruits) and not on South African prickly acacia (V. nilotica subsp. kraussiana [Benth.] Kyal. & Boatw. with non-moniliform fruits), indicating a high host plant specificity. Based on these results, a colony of the gall thrips from Ethiopia was established in a high security quarantine facility in Brisbane, Australia in December 2015 and host specificity tests are in progress. To date, no-choice tests have been completed for 50 test plant species, and thus far, the thrips have only induced galls on subsp. indica from Australia and subsp. tomentosa from Ethiopia (both with moniliform fruits).

Gall midge

In Ethiopia, a gall midge morphologically similar to Lopesia niloticae Gagné (Diptera: Cecidomyiidae) induced leaf rachis galls (Figure 3c) on all three subspecies of V. nilotica: subsp. indica (61% of sites, n = 14), subsp. tomentosa (83% of sites, n = 20) and subsp. leioarpa (63%, of sites, n = 5). A morphologically
Figure 3. Gall-inducing insects and mites associated with Vachellia nilotica in Africa. Shoot-tip rosette galls induced by the gall thrips Acaciothrips ebneri (a) in Ethiopia and (b) in Senegal; (c) rachis and leaflet gall induced by the gall midge Lopesia niloticae in Ethiopia; (d) red spherical leaflet mite gall (type-1) induced by Aceria sp. 1 in Ethiopia; (e) creamy-white fluted leaflet mite gall (type-2) induced by Aceria sp. 2 in Ethiopia; leaflet, rachis and shoot-tip deforming mite gall (type-3) induced by Aceria sp. 3 (f) in Ethiopia and (g) in Senegal; (h) stem galls induced by the tephritid gall fly Notomma mutillum in Senegal.
similar gall midge has also been seen on other co-
occuring *V. abyssinica* (*n* = 7 sites) and *V. etbaica*
(*n* = 4 sites) trees in Ethiopia. There was no evidence
of the gall midge on the two subspecies of *V. nilotica*
in Senegal.

**Gall mites**

Three morphologically distinct *Aceria* gall mites
were found on *V. nilotica* subspecies in Ethiopia: red
spherical leaflet galls (type-1; Figure 3d), creamy-
white fluted leaflet galls (type-2; Figure 3e) and hairy
mushroom-like galls on leaflets, rachides and shoot-
tips (type-3; Figure 3f). Type-1 (*Aceria* sp. 1) leaflet
galls (Figure 3d) were seen on all three subspecies:
subsp. *leiocarpa* (55% of sites; *n* = 4), subs. *tomentosa* (67% of sites, *n* = 16) and subsp. *indica* (56% of sites, *n* = 13). Type-2 (*Aceria* sp. 2) leaflet galls (Figure 3e)
were seen only on subsp. *leiocarpa* (75% of sites, *n*
= 6) and not on subsp. *tomentosa* (*n* = 24 sites) or
subsp. *indica* (*n* = 23 sites). Type-3 (*Aceria* sp. 3)
galls on leaflets, rachides and shoot-tips (Figure 3f)
were found only on subsp. *tomentosa* (46% of sites, *n*
= 11) and subsp. *indica* (57% of sites, *n* = 13) and
not on subsp. *leiocarpa* (*n* = 8 sites). Both type-1 and
type-3 galls were often found on the same leaves.
Galls morphologically similar to the three mite galls
found on *V. nilotica* were not seen on co-occurring
*V. abyssinica* (*n* = 7 sites) and *V. etbaica* (*n* = 4 sites)
trees.

In Senegal, type-2 creamy-white fluted leaflet
galls were found only on subsp. *adstringens* in all sites
(*n* = 8 sites), and type-3 hairy mushroom like galls
deforming leaflets and rachides (Figure 3g) were
found on subsp. *tomentosa* (25% of sites, *n* = 2) and
subsp. *adstringens* (50% of sites, *n* = 6). There was
no evidence of morphologically similar mite galls in
co-occurring *V. seyal*, *V. tortilis* and *S. senegal* trees.

A colony of type-3 gall mites from Ethiopia
was established on subsp. *indica* (sourced from
Australia) in the quarantine facility at PHP, South
Africa in December 2017. To date, no-choice tests
have been completed on *V. nilotica* subsp. *krassiana*,
*V. nilotica* subsp. *adstringens*, *V. nilotica* subsp.
*tomentosa*, *V. sideriana* (DC.) Kyal. & Boatw., *V.
hebeclada* (DC.) Kyal. & Boatw., *Senegalia galpinii*
(Burtt Davy), and *Paraserianthes lopanthoa* (Willd.)
I. C. Nielson. The type-3 gall mite has induced galls
only on subsp. *indica* sourced from: Australia (11
± 5.4 galls per plant) and host specificity testing is
ongoing for remaining test plant species.

**Gall fly**

A stem gall-inducing *Notomma mutilum*
(Bezzi) (Diptera: Tephritidae) (Figure 3h) has been
identified as a prospective agent from Senegal. This
is the first time a gall-inducing tephritid associated
with *V. nilotica* has been collected. The gall fly
was found in 86% of surveys sites (*n* = 20 sites) in
Senegal, on both subsp. *tomentosa* (*n* = 12 sites) and
subsp. *adstringens* (*n* = 8 sites), but not on other co-
occuring *Vachellia*, *Acacia* and *Senegalia* species (*n*
= 17 sites). The number of galls per: shoot ranged
from 1 to 12. There was no evidence of the gall fly
in Ethiopia.

The gall fly was imported from Senegal, into
quarantine in Brisbane, Australia, for colony
establishment and host specificity testing. In October
2017, over 800 stem-cuttings with stem galls of *N.
mutilum* were imported, but no adults emerged from
this material. In April 2018, about 600 stem-cuttings
with stem galls of *N. mutilum* were imported again
from Senegal, and about 240 adults emerged from
this second importation. Potted prickly acacia plants
(subsp. *indica*) were exposed to newly emerged
adults in insect-proof cages for oviposition and
colony establishment.

Adult flies live up to four weeks under quarantine
conditions. The adults lay eggs near the meristem
of new shoot growth, and the emerging larvae enter
through the meristem and induce stem galls. Stem
gall development is evident after two weeks. The life
cycle from adult to adult is from 4 to 5 months.

**Discussion**

The close affinity of prickly acacia to the
Australian native *Vachellia* and *Acacia* species
makes the biological control efforts difficult. Any
potential biological control agent for prickly acacia
in Australia needs to be species specific, with no
significant risk to Australian native species. Hence,
priority was given to gallling agents, in view of their
likely host specificity. Based on field host range,
geographic range and damage levels, the gall thrips
and the type-3 gall mite, both from Ethiopia, and the tephritid gall fly from Senegal have been prioritized for detailed host specificity tests.

Field host range in Ethiopia and preliminary host specificity tests in South Africa suggest that the gall thrips is host specific, with its host range restricted to *V. nilotica* subspecies with moniliform fruits (Dhileepan et al., 2018). This is supported by ongoing no-choice host specificity tests in quarantine in Australia; so far the thrips have induced galls only on prickly acacia. Host specificity tests are continuing.

The leaflet and rachis midge galls from various *V. nilotica* subspecies in Ethiopia and from *V. abyssinica* in Ethiopia are yet to be identified, though they appear similar to the gall midge (*L. niloticae*) on subsp. *leioarpa* in Kenya. Phytophagous Cecidomyiidae generally have narrow host-plant ranges, and therefore, in Cecidomyiidae taxonomy, species distinctions are often made on the basis of host association (Gagné 1986). However, it is essential to ascertain the taxonomy of the mites from various subspecies of *V. nilotica*, before future work on the gall midge is considered.

The eriophyd mites (*Aceria* spp.) inducing the three morphologically distinct galls in Ethiopia appear very similar to *Aceria liopeltus* Meyer (Acari: Eriophyidae) inducing leaflet galls on subsp. *kraussiana* in South Africa (Charnie Craemer, pers. comm.). In both Ethiopia and Senegal, type-1 and type-3 mite galls were often found on the same leaf, suggesting that the distinct gall morphologies are not host related and are likely caused by different gall mite species. In tests in South Africa, gall mite *A. liopeltus* sourced from subsp. *kraussiana* induced galls on subsp. *kraussiana* and not on subsp. *indica* (Witt 2004, Dhileepan et al., 2014). The type-3 gall mite from Ethiopia induced galls on subsp. *indica* from Australia and subsp. *tomentosa* from Ethiopia (both with moniliform fruits) and not on subsp. *tomentosa* from Senegal (with moniliform fruits), subsp. *astringens*, subsp. *kraussiana* and subsp. *leioarpa* (all with non-moniliform fruits). This indicates that the gall mites are host specific at the subspecies level. Though morphologically indistinguishable from *A. liopeltus* inducing galls on subsp. *kraussiana*, field host specificity and gall morphology suggest that the mites inducing the three gall types in Ethiopia and Senegal are likely to be distinct species. Future research will focus on using molecular tools in resolving the species status of the mites inducing the three types of galls.

The type-3 gall mite from Ethiopia was prioritized for host specificity testing based on gall morphology, field host range and damage potential. To date there has been no gall development on any non-target plant species. If proven host specific in the preliminary host specificity tests, type-3 gall mite will be imported to a high security quarantine in Australia for detailed host specificity tests.

*Notomma* Bezzi is a genus of eight described species of tephritid gall fly predominantly from Africa (Munro, 1952; Freidberg and Murgolis 2011). All known hosts are from the Family Fabaceae and species are known to be restricted to a single host species (Hancock 1986; Munro, 1952; Stefan Nesen, pers. comm.). This is the first time a tephritid gall fly has been recorded on *V. nilotica* in Senegal. Very little is known about this fly or its biology, as only two specimens had ever been collected before this study (M.W. Mansell, pers. comm.). In Senegal, the tephritid gall fly was found only on *V. nilotica*, with no evidence of the gall fly on other co-occurring *V. seyal*, *V. tortilis* and *S. senegal* species at the survey sites, suggesting that the gall fly is likely to be host-specific. Host specificity tests for the gall fly will commence once a colony has been established in quarantine in Brisbane, Australia.

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