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## Short communication

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### First report on *Chenopodium quinoa* Willdenow (Amaranthaceae) stem-boring damage by *Athesapeuta dodonis* (Marshall) (Coleoptera: Curculionidae) with associated fungal spp. interactions in South Africa

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Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal originating from the Andean mountain chain where diverse biotypes with cold and drought tolerance traits exist. Introduction of this hardy annual into South Africa is very recent and information on associated pest and disease complexes is limited for the southern African subcontinent. Recent observations made on *C. quinoa* plant damage caused by *Athesapeuta dodonis*, are highlighted. A description and illustrations of damage symptoms caused by this weevil are given. *Athesapeuta dodonis* also attacks other Amaranthaceae in South Africa, which necessitates an approach that includes perspectives on damage symptoms previously described for *Amaranthus* spp. In addition, and because of the close phylogenetic relationship between these plant genera, plant damage symptoms are also viewed from the perspective of the large South African weevil complex that exists on *Amaranthus* spp. Comparisons in terms of similarities between possible weevil fungal associations described earlier for *Amaranthus* spp. are drawn for *C. quinoa* and dominant fungal taxa are highlighted.

*Chenopodium quinoa* is a hardy annual originating from the Andean mountain chain where it grows from sea level to 4000 m above sea level (Jacobsen 2003). It possesses a high level of genetic plasticity and has adaptation for cold, drought and soil salinity tolerance spread to a varying degree across numerous ecotypes. The recent rise in *C. quinoa* production relates to global expansion beyond traditional Bolivian and Peruvian production areas, which still are responsible for the bulk of grain sold in the world. According to Bazile *et al.*

(2016) the rate of expansion has accelerated with numbers jumping from eight countries growing *C. quinoa* during 1980 to 40 by 2010 and 75 by 2014 with an additional 20 countries of first introduction during 2015. Production and/or research currently resides throughout Africa, Europe, Asia and the South American Andean production zones, as well as parts of the U.S.A., Canada and the United Arab Emirates. Development of *C. quinoa* varieties has also been ongoing in Russia since 2009 (Shmatkova 2019).

The surge in global demand for *C. quinoa* may firstly be attributed to its high protein quality, which is underscored by high percentages of essential amino acids including lysine (defined as a limiting amino acid), and secondly for the absence of gluten in its grain (Navruz-Varli & Sanlier 2016). *Chenopodium quinoa* is functionally classified as a pseudocereal and is closely related to plants in the genus *Amaranthus* L. Both genera are classified within the family Amaranthaceae (order Caryophyllales) (Tahmasebi & Firuzkoochi 2017) within which *Amaranthus* hosts three species *viz.* *A. hypochondriacus* L., *A. caudatus* L. and *A. cruentus* L. Mentioned species are globally acknowledged as grain types, and collectively the source of 'the other' type of Andean/Central American pseudocereal. Pseudocereal interests in South Africa currently include both genera, which possess very similar seed morphological and nutritional traits.

No knowledge base exists for *C. quinoa* production under South African conditions. Serious attempts to evaluate and multiply *C. quinoa* were only made from as recent as the 2013/14 growing season. Most of these efforts have been limited to



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greenhouse environments and enclosed restricted areas due to a lack of legislation for introduction of *Chenopodium* spp. To the contrary, *Amaranthus* spp., cultivated for either leaf or grain production, has been much more extensively researched in South Africa and has a knowledge base that pre-dates *C. quinoa* by approximately two decades. Given the close phylogenetic relationship that exists between these two crops (Kadereit *et al.* 2003), a better understanding of *C. quinoa* pests and diseases may be gained by viewing findings from within the perspective of a more extensive *Amaranthus*-centred data base.

The initial research thrust involving a potential pseudocereal crop in South Africa was drawn toward leaf production potential of *Amaranthus* spp. Early surveys conducted by Louw *et al.* (1995) revealed seven associated Curculionidae species from five central South African localities, which at the time, already surpassed Curculionidae diversity and niche occupation reported on in global literature. During the latter study, *Ath. dodonis* (Marshall) (Curculioninae: Baridini) (previously referred to as *Baris dodonis* Marshall) was reported along with *Baris amaranthi* Marshall and an unidentified *Baris* sp. as potentially important endophytic feeders along with *Hypolixus haerens* (Boheman), *Gasteroclisus cuneiformis* (Fahraeus) and *Neocleonus sannio* (Herbst) (Curculioninae: Lixini). The species count for Curculionidae sampled on *Amaranthus* spp. has since increased to at least nine species (pending identification of additional Baridini) (Weeks & Louw 2013). This increase comes in spite of a decreased pest status ranking for *N. sannio*, which was one of the first weevil species reported. It is currently considered as only of minor significance on *Amaranthus* spp. (Prinsloo & Uys 2015) due to highly polyphagous opportunistic feeding habits. *Athesapeuta dodonis* eggs, larvae, pupae and adults were sampled from *Amaranthus* spp. stems. Larvae were shown to also bore into taproots Louw *et al.* (1995). These findings highlighted the importance of the Curculionidae as amaranth pests. To the contrary, Curculionidae citations are relatively scarce from literature reviewing the pest complex in native *C. quinoa* production areas. A review done by Rasmussen *et al.* (2003) identified two Lepidoptera spp., viz. *Eurysacca melanocampta* (Meyrick) and *E. quinoae* Povolný (Gelechiidae), as the most serious pests of this crop in Andean production areas. An *Adioristus* sp. is listed as the only Curcu-

lionidae species sampled from *C. quinoa* in Peruvian and Bolivian production areas.

Blodgett *et al.* (2000) reported on the first study to document endophytic fungi associated with asymptomatic leaves, petioles, stems and roots sampled from *Amaranthus hybridus* L. Field establishment of *A. hybridus* at Potchefstroom (26°42'S 27°6'E) and Bloemfontein (29°01'S 26°08'E) revealed geographical and plant organ-based shifts in fungal species composition with a general pattern of domination of *Alternaria* spp. within leaves and petioles and *Fusarium* spp. (mainly *F. oxysporum*) in roots. Attention was given towards understanding potential interactions between stem-boring weevils and fungi. Louw *et al.* (2002) revealed weevil × pathogenic fungal associations for the first time on *A. hybridus*. *Athesapeuta dodonis*, *H. haerens* and *Microlarinus angustulus* Marshall (Curculioninae: Lixini) were also found in association with *Fusarium* spp., with *Ath. dodonis* and *H. haerens* considered most injurious in this regard. Few reports on global *C. quinoa* disease occurrence are available, and a study of *C. quinoa* diseases would be invaluable to the future establishment of a *C. quinoa* industry in South Africa.

The expansion of *Amaranthus* leaf-oriented research to include primarily grain-oriented production, necessitated a re-evaluation of the relative pest status of all known amaranth insect pests (Weeks & Louw 2013). *Chenopodium quinoa* introduction and value chain development is centred on grain production, which is unlikely to change when considering the current global perspective. This report confirms *Ath. dodonis* as a stem-boring pest of *C. quinoa* in South Africa, with subsequent descriptions of damage symptoms as well as preliminary observations on parasitoid and fungal associations.

*Chenopodium quinoa* was sown during late May 2018 (austral winter) inside an open plant growth tunnel at the Department of Agriculture and Rural Development's (DARD) Potchefstroom based research farm. Sowing was aimed at seed multiplication and was done in 5-l plastic bags containing a mixture of 37.5 % fine sand, 37.5 % building sand, 12.5 % coarse river sand and 12.5 % Hygromix growing medium. Plants were first inspected during August 2018, and removed for dissection during early December 2018. Main stems, shoots and root crowns of 20 randomly selected plants were cut longitudinally to view stem-borer damage symptoms and collect insect life stages.

Intact stems of a further 20 randomly selected plants were placed in a 25-l plastic container and sealed to prevent escape of emerging insects. Samples of all insects that emerged were collected from the container on a weekly basis.

Individually dissected plant root sections, stems, petioles and inflorescences were randomly collected and surface sterilised in a 7 % NaOCl solution for 5 min. Plant parts were rinsed in distilled water for 5 min prior to plating out on Potato Carrot Agar (PCA) and Selective Fusarium Agar (SFA). Frass collected from stem galleries was plated on PCA and SFA, respectively. Larvae and pupae were placed in sterile Eppendorf tubes immediately after collection and euthanased at  $-80^{\circ}\text{C}$  for 2 h (Vermeulen *et al.* 2017). Half of larvae and pupae collected were surface sterilised and plated on PCA and SFA (both intact and macerated). Maceration of larvae enabled isolations of fungi from the alimentary canal to be made. The remaining half of larvae and pupae collected were plated out directly without surface sterilisation. Plates were kept at  $25^{\circ}\text{C}$  for 5 days before fungal colonies were purified and single spored prior to identification based on morphology.

Large numbers of *Ath. dodonis* adults (Fig. 1) were recorded in *C. quinoa* stems and on inflorescences during early development of panicles, anthesis and grain filling. This indicates the possibility of constant presence of all life stages on *C. quinoa* plants prior to damage symptom development for at least the duration of reproductive growth phases. Similar to *Amaranthus*, feeding was observed for *Ath. dodonis* adults on *C. quinoa* panicles during greenhouse production cycles in previous seasons. First observations of severe plant damage symptoms, which included stunting concomitant with stem boring (Fig. 2) were, however, only made towards August (austral spring) of 2018. Relative Pest Status Indices (RPSI) calculated for amaranth grain production by Weeks & Louw (2013) (based on RPSI proposed by Moran 1983) rated *Ath. dodonis* third, in terms of overall injuriousness potential, with an overall score of 15 compared to 15.5 for *H. haerens* and 16.5 for the facultative seed feeder *Nysius natalensis* (Schilling) (Heteroptera: Orsiliidae). Recent observations on *C. quinoa* damage extends niche occupation for *Ath. dodonis* to more closely resemble niche occupation observations made on *Amaranthus* spp.

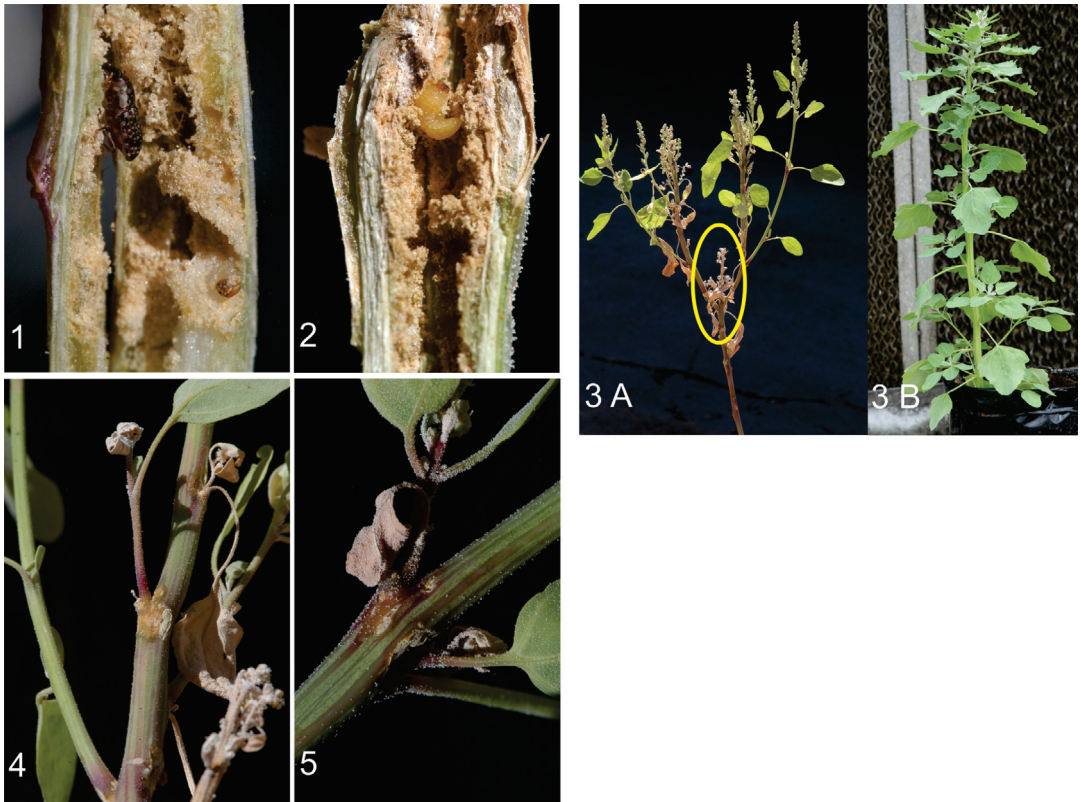
Main stem development was severely impaired

in all plants (Fig. 3A), and were subject to stunting, necrosis and eventually total die-back. All main stems initiated primary inflorescences, which remained rudimentary in all plants inspected due to early arrestment of development (Fig. 3A and Fig. 3B). The resulting lack of apical dominance on infested plants led to a proliferation in side shoot development (Fig. 3A), which in turn was impacted by continuous re-infestation that occurred during most of the growing period following initial infestations. External symptoms on stems included discolouration which was evident at axils where branching of secondary stems occurred (Figs 4 and 5). Stems appeared asymptomatic on the inside except for extensive tunnelling and galleries filled with frass (Fig. 2). Asymptomatic internal stem damage on *C. quinoa* deviates from the clearly defined dark discoloured stem cancers described by Vermeulen *et al.* (2018), found in association with *Ath. dodonis* and *B. amaranthi* emergence hole proliferation on *Amaranthus* spp. Tunnelling was not observed to extend into roots in any of the plants investigated.

Adults from two unknown parasitoid genera (Hymenoptera: Braconidae and Braconidae: Cheloninae, respectively) as well as an *Entedon* sp. (Hymenoptera: Eulophidae) were reared from infested stems. *Entedon* spp. are considered host specific parasitoids of weevils (Louw *et al.* 1995).

The *Fusarium oxysporum* spp. complex comprised the dominant fungi isolated from plant organs and *Ath. dodonis* larvae and pupae. Second in terms of species dominance were *Fusarium* spp. classified outside the *F. oxysporum* spp. complex (Table 1). The highest frequency of fungal isolations was recorded from stems, asymptomatic petioles, *Ath. dodonis* larvae, pupae and frass (Table 1). These observations were made irrespective of whether or not surface sterilisation was done.

The rapid globalisation of *C. quinoa* production provides simultaneous access to areas of truly ancient cultivation, areas of medium to short-term cultivation (more than 20 years but less than 100 years) and areas of very recent introduction (less than 10 years). This provides a truly unique opportunity for comparative studies on the dynamics of global pest complex development. Introduction of crops into areas of no previous cultivation creates a knowledge vacuum. In such instances subsequent success in production establishment will depend on the capacity to generate



**Figs 1–5.** 1, *Athesapeuta dodonis* adult inside *Chenopodium quinoa* stalk. 2, *Athesapeuta dodonis* larvae within main stem gallery with slight tissue discolouration. 3A, Secondary stem proliferation on severely infested plant. 3B, Healthy *Chenopodium quinoa* plant. 4, Shoot and leaf necrosis linked to *Athesapeuta dodonis* stem infestation. 5, External discolouration and distortion of stem axil, shoots and petioles.

knowledge critical for strategic decision making. In the absence of such knowledge production outcomes become uncertain, which limits access that producers have to important resources such as funding and logistic support.

Current and very recent insight into *Ath. dodonis* niche occupation (and possibly recent niche expansion) should not be considered as complete, because the nature and extent of inflorescence feeding by this weevil must still be described with greater accuracy. Inflorescences do provide several options for niche proliferation in the form of flowers, pollen and/or seed feeding, which are linked to plant phenology by becoming available over a temporal scale. In addition, the pericarp of mature *C. quinoa* seed contains saponins (Jancurová *et al.* 2009), which may act as feeding deterrents, at least mediated through anti-xenosis. The impact this will have on the biting chewing feeding guild (which includes weevils) during the

course of seed development on plants must still be explored further.

*Athesapeuta dodonis* is considered to be polyphagous, occurring on most *Amaranthus* spp. and also on *Chenopodium album* L. (Amaranthaceae), which was considered to be its primary host by the late S. Louw (unpubl.). Adults of this weevil were also found more recently on *Beta vulgaris* (L.) (Amaranthaceae), which was grown at the Dithakwaneng Village in the Naledi District of the North-West Province of South Africa; sampled by W.J. Weeks (1 November 2016). The exact nature of association and possibility of damage on this vegetable crop is still unclear. Resolving of taxonomic issues within the Caryophyllales may also impact on the status of *Ath. dodonis*. Should the current botanical classification be upheld, it might be argued that *Ath. dodonis* should be considered an oligophagous rather than a polyphagous pest, associated with the Amaranthaceae.

**Table 1.** Summary of fungal isolation frequency linked to fungal taxa and loci sourced.

| Source of Isolation                          | Total number of isolations | Number of fungal spp. isolated | Fungal species  |
|--|----------------------------|--------------------------------|---|
| Roots  | 16                         | 8                              | <i>Penicillium</i> sp. (1)<br><i>Trichoderma</i> sp. (1)<br><i>Fusarium oxysporum</i> spp. complex (3)<br><i>Phoma</i> spp. (3)                                     |
| Root and crowns                              | 16                         | 6                              | <i>Trichoderma</i> sp. (1)<br><i>Fusarium subglutinans</i> (2)<br><i>Fusarium oxysporum</i> (1)<br><i>Phoma</i> sp. (1)   |
| Stems  | 12                         | 12                             | <i>Fusarium oxysporum</i> spp. complex (12)   |
| Asymptomatic petioles                        | 12                         | 12                             | <i>Fusarium oxysporum</i> spp. complex (12)   |
| Asymptomatic panicles                        | 18                         | 7                              | <i>Fusarium oxysporum</i> spp. complex (7)  |
| Larval frass (surface sterilised galleries)  | 10                         | 7                              | <i>Fusarium oxysporum</i> spp. complex (7)  |
| Larval frass (non-sterilised galleries)      | 16                         | 16                             | <i>Ascochyta</i> sp. (1)  |
| Larvae (intact and surface sterilised)       | 6                          | 6                              | <i>Fusarium oxysporum</i> spp. complex (2)<br><i>Fusarium</i> spp. (other than <i>F. oxysporum</i> spp. complex) (13)<br><i>Fusarium oxysporum</i> spp. complex (6) |
| Larvae (macerated and surface sterilised)    | 4                          | 4                              | <i>Fusarium oxysporum</i> spp. complex (1)<br><i>Fusarium</i> spp. (other than <i>F. oxysporum</i> spp. complex) (3)  |
| Larvae (intact and non-surface sterilised)   | 8                          | 8                              | <i>Fusarium oxysporum</i> spp. complex (6)<br><i>Fusarium</i> spp. (other than <i>F. oxysporum</i> spp. complex) (2)  |
| Pupae (intact and non-surface sterilised)    | 1                          | 1                              | <i>Fusarium oxysporum</i> spp. complex (1)  |
| Pupae (macerated and non-surface sterilised) | 1                          | 1                              | <i>Fusarium oxysporum</i> spp. complex (1)  |

The final pattern for production cycles, crop seasonality and geographical production regions has by no means been established for *C. quinoa* in South Africa. Current indicators suggest a shift in production cycles towards the austral autumn and winter months for most parts of central South Africa should production of scale ever take hold. Altitude shifts linked to geographical production region shifts will however further impact on crop seasonality, and will undoubtedly also impact on pest dominance, injuriousness and diversity. Latest observations and the corresponding severity of associated symptoms suggest *Ath. dodonis* to be one of the dominant pests on *C. quinoa* in South Africa. Final pest status will however only become clearer once best agronomical practice (including production and rotation cycles), integrated pest management strategies, geographical and climatologically dictated crop distribution patterns, and the development of industry support for production, has been established. Expected temperature increases during austral autumns and winters, which are associated with climate shift patterns (Kurukulasuriya *et al.* 2006), may further contribute to the injuriousness of this pest and its proficiency as a potential disease vector.

The *F. oxysporum* spp. complex was isolated from all plant organs sampled, as well as from *Ath. dodonis* larvae, pupae and frass. This corresponds with persistent *Fusarium* dominance in fungal isolations made from *Ath. dodonis* adults

sampled from *A. cruentus* by Vermeulen *et al.* (2018). Highest infection rates were within stems and asymptomatic petioles, which correspond with fungal isolation frequencies on *Ath. dodonis* larvae, pupae and frass (regardless of whether or not surface sterilisation was performed). This would support the *A. cruentus* observation-based hypothesis (Vermeulen *et al.* 2018) that *Ath. dodonis* may vector *Fusarium* spp. *via* specific ingestion of or transporting of spores *via* the integument. The exact nature of the association between *Ath. dodonis* and *Fusarium* spp. in relation to infection patterns on *C. quinoa* will however have to be investigated in more detail. Better understanding of *Fusarium* spp. interactions with *Ath. dodonis* on *C. quinoa* and related Amaranthaceae such as *C. album* and *Amaranthus* spp. (which occur globally and throughout the North-West Province as weeds and/or crops) will be important for Integrated Pest Management (IPM) strategy development in future pseudocereal production. A better understanding of tri-trophic interactions is necessary for IPM development. This is so especially because parasitoids appeared unable to maintain *Ath. dodonis* populations below damage threshold levels during the recently observed infestations on plants emerging from a winter growth cycle.

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