# A new highly virulent bluegreen aphid causes severe damage in previously tolerant pasture and grain legumes

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## Abstract

Bluegreen aphid (BGA, *Acyrthosiphon kondoi*) has recently been observed to cause unusually severe damage and mortality in seedlings of previously tolerant pasture legume cultivars. In 2009, preliminary greenhouse screening suggested that the observed symptoms were the result of a more virulent BGA biotype that if widespread would have the potential to devastate pasture establishment and production.

*Medicago* spp. (lucerne and annual medics) and annual *Trifolium* pasture legumes appear to be sensitive hosts, with previously tolerant cultivars exhibiting only small increases in relative tolerance compared to intolerant cultivars. Tolerance was overcome in over ten species and 40 cultivars evaluated.

Severe damage and mortality was found in lentils and narrow leaf lupins. Other grain legumes also host the new BGA but are moderate to highly tolerant, including (in order of increasing tolerance) albus lupins, faba beans and field peas.

The new BGA remains susceptible to insecticides. In greenhouse experiments, chemical seed treatment with imidacloprid protected young lucerne seedlings for four weeks after emergence. Farmers and agronomists should be vigilant with regular monitoring for aphid damage, which may be particularly severe on newly sown (or regenerating) pastures and grain legumes.

## Introduction

Bluegreen aphids (*Acyrthosiphon kondoi* or BGA) have been a major widespread pest of legume pastures in Australia since their discovery in 1979 (Milne, 1986). They are a relatively large aphid (up to 3 mm long) with a matte bluegreen colour that feed on the upper leaves, stems and terminal buds of host plants. BGA cause damage to plants by direct removal of nutrients, secretion of bioactive compounds (from their saliva) into the plant, transmission of plant pathogenic viruses, and secretion of honeydew that can cause secondary fungal growth and inhibit photosynthesis (Edwards 2008).

The SARDI pasture breeding programs have been producing aphid-tolerant lucerne and annual medic cultivars for 30 years. Resistance to BGA is mediated through the phloem, requiring an intact plant and involving a combination of antixenosis (the inability of the plant to serve as a host), antibiosis (a response from the plant that is detrimental to the aphid) and plant tolerance (Klinger et al. 2009). In California, BGA resistance in lucerne was broken down by a new BGA biotype discovered almost 20 years ago (Zarrabi 1995). Previously tolerant lucerne germplasm provided no greater protection than the susceptible control. Populations of aphids in Australia are also known to be genetically variable. A study by Mackay and Lamb (1988) identified 3-7 genetically different clones from a collection of 12 samples in a single paddock. The aphid clones differed in life history traits that included survival, fecundity, growth-rates and percentage of winged aphids. A further study by Edwards (2001) showed variation in the growth rates of BGA clones collected in Western Australia on a range of grain legumes including narrow leaf lupin (*Lupinus angustifolius* L.), faba bean (*Vicia faba* L.), field pea (*Pisum sativum* L.), and lentils (*Lens culinaris* Medikus). Plant responses to the different BGA populations however, were not measured in either of these studies. A routine part of the SARDI BGA screening program is to collect new BGA populations from *Medicago* spp. in the field each year. Aphids collected from a source close to the Waite Institute in April 2009 had much greater virulence on all previously tolerant lucerne and annual pastures, producing high rates of plant mortality. This paper presents initial results from characterising the tolerance of a broad range of pasture and grain legumes to this potential new BGA biotype.

## Methods

A series of four experiments (Table 1) were performed to evaluate the tolerance of a range of pasture and grain legumes to a putative new biotype of BGA. We screened a wide range of lucerne cultivars from Australian and international breeding programs, and BGA tolerant annual medic cultivars with diverse geographical origin. Plants were grown in ideal conditions for aphid culture, at 24?C with a 16 hour day length. Plants were inoculated at a rate of 2 aphids per plant one week after planting when the plants had fully extended cotyledons. Plants were visually assessed for damage and scored on a 1 to 5 scale (1 = no visual damage to 5 = plant dead) after 4 weeks as per the method published by Nair *et al.* 2003. Plants with a rating of 1 or 2 are considered tolerant. Lucerne, annual medics and sub clover were planted at 50 seeds per pot and the grain legumes with their larger seed size were planted at 4 seeds per pot. Treatments were replicated four times.

A further experiment was conducted to determine the effect of imidacloprid seed treatment on damage caused by BGA on the previously tolerant SARDI Seven lucerne cultivar. This experiment followed the same protocol as experiments 1-3, except that each experimental unit was housed in an individual aphid-proof tent to eliminate cross contamination of BGA, and 50 seeds were planted in each of 12 pots to make up an experimental unit (600 seeds per replication).

**Table 1. Details of experiments conducted to investigate increased virulence of potential new bluegreen aphid biotype.**

| Experiment | Species and expected BGA response | Cultivars |
|---|---|---|
| 1. Lucerne | Lucerne (*Medicago sativa*): Tolerant | Cropper 9.5, Force 10, Hallmark, Icon, Pegasus, SARDI Five, SARDI Seven, SARDI Ten, Saturn, Stamina GT6, WL925HQ |
| ? | Lucerne (*Medicago sativa*): Intolerant | Arc |
| 2. Annual pasture legumes | Medics (*Medicago* spp.): Tolerant | *M. truncatula*: Caliph, Jester, Mogul, Parabinga, Paraggio, Sephi. *M*. *littoralis*: Angel, Herald. *M. tornata*: Toreador. *M .rugosa*: Paraponto. *M. scutellata*: Kelson |
| ? | Medics(*Medicago* spp.): Intolerant | *M .truncatula*: Borung, Jemalong. *M*. *littoralis*: Harbinger. *M. polymorpha*: Cavalier, Santiago, Scimitar. *M .tornata*: SA8645 |
| ? | Clovers (*Trifolium* spp.): Moderately tolerant | *T. subterraneum*: Antas, Gosse, Mintaro. *T. michelianum*: Frontier |
| 3. Grain legumes | Albus lupin (*Lupinus albus*),<br><br>Narrow leaf lupin (*Lupinus angustifolius* L.), faba bean (*Vicia* | Albus lupins: Luxor, Rosetta. Narrow Leaf Lupin: Jendabilup, Jindalee, Mandelup, Moonah. Field Pea: Celine, Kespa, Moonlight, Parafield, Santi, Yarrum. Faba bean BL15/1, Farah, Fiord, Nura. |

| | | |
|---|---|---|
| | *faba* L.), field pea (*Pisum sativum* L.), lentils (*Lens culinaris*) and chickpea (*Cicer arietinum*) | Lentil: Boomer, Northfield. Chickpea: Almaz, Genesis090 Genesis509. |
| 4. Imidacloprid coated seed | Lucerne (*Medicago sativa*) | SARDI Seven |

**Results**

*Experiments 1 and 2*

All *Medicago* and annual *Trifolium* cultivars evaluated were found to be intolerant hosts to the putative new BGA, with zero plant survival observed 4 weeks after inoculation with BGA (Table 2). Intolerant *Medicago* cultivars had aphid damage scores of 4 and 5 by day 11 while tolerant medic cultivars and sub clovers cultivars had most plants with damage score of 2. However, over the next 5-7 days the tolerant *Medicago* and sub-clovers rapidly developed damage and then died (rating 5).

**Table 2. The putative new BGA biotype resulted in the death of all annual medic, lucerne, sub clover and balansa clover plants.**

| Group | Expected % Tolerant | Observed % Tolerant | Expected % Survival | Observed % Survival |
|---|---|---|---|---|
| Intolerant annual medics | 0 | 0 | <30 | 0 |
| Tolerant annual medics | 100 | 0 | 100 | 0 |
| Clovers (balansa, sub-clover) | 100 | 0 | 100 | 0 |
| Intolerant Lucerne (arc) | 0 | 0 | >95 | 0 |
| Tolerant Lucerne | 30-50 | 0 | >95 | 0 |

*Experiment 3*

All grain legumes with the possible exception of chickpea were able to host the putative new BGA. Narrow leaf lupins and lentils were the most severely effected with a high percentage of plant mortality in these crops (rating above 4). Field peas previously thought to have some damage from BGA were shown to be quite tolerant in this experiment; however one late germinating plant was killed by a high population of BGA. Faba beans and albus lupins exhibited moderate-high tolerance, with variability within cultivars observed indicating the possibility of segregation for BGA tolerance.

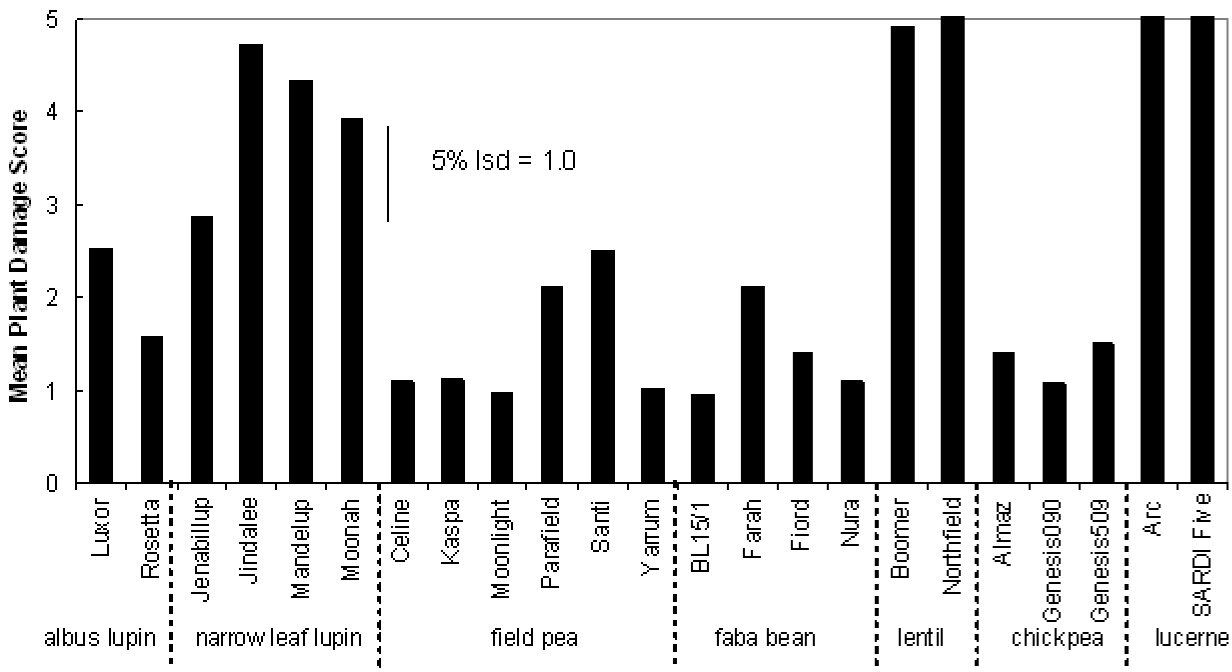


**Figure 1: Mean plant damage score of grain legume cultivars screened with the new BGA.**

*Experiment 4*

Treating lucerne seed with the insecticide imidacloprid dramatically increased the percent of plants with a damage score of less than 3 and increased dry weight yield (figure 2).

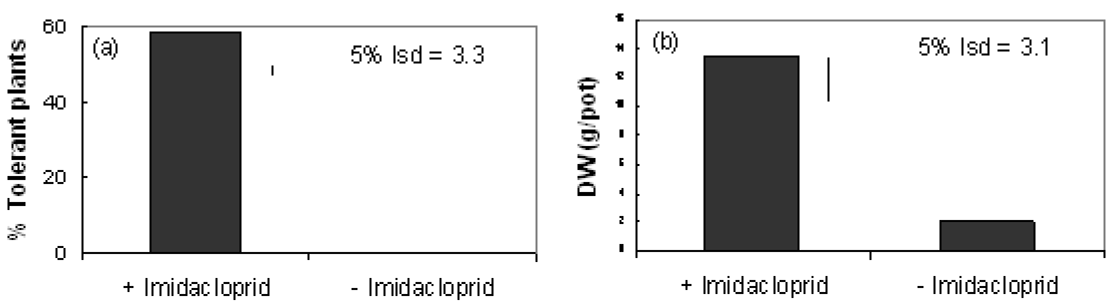


**Figure 2. Imidacloprid insecticide applied to seed coat of SARDI Seven lucerne cultivar increases (a) tolerance (% of plants with damage score of 1 or 2) and (b) dry weight yield after 4 weeks.**

**Discussion**

The population of BGA that was collected in 2009 has proven to be much more virulent on tolerant cultivars in our glasshouse screening trials than BGA populations collected prior to 2009. Indeed, the 2009 BGA resulted in the death of all pasture legume cultivars tested. All lucerne plants were killed whereas prior populations of BGA only caused severe stunting that rarely resulted in plant death. For tolerant cultivars of lucerne we expect 30-50% of plants to show little or no damage, however with the 2009 BGA population all cultivars tested showed 100% mortality. For tolerant cultivars of annual medics we expect plants to have uniform damage scores of 1 or 2 however with the 2009 BGA, all plants died (score 5). Nair et al. 2003 reported scores near 3 for sub and balansa clover cultivars in response to BGA. With the 2009 BGA, sub and balansa clover all died. On the basis of these observations we believe we have discovered a new highly damaging BGA biotype (new BGA).

In the legume crops, the severe damage and high mortality in lentils and narrow leaf lupins due to BGA is a major concern for grain legume production in Australia. Whilst the authors have not previously evaluated BGA tolerance in grain legumes, it appears to represent a significant increase over previous reports of tolerance of these species. For example, Edwards (2001) found that lentils (cv. Digger) and narrow leaf lupins (cv. Tallerack) did not support BGA population growth at a level similar to that observed for green peach aphid and cowpea aphid. In this study, rapid aphid population growth (followed closely by mortality) was observed on these hypersensitive hosts. Damage in legume crops may also be linked to growth stage, as late emerging seeds of field peas and albus lupins were killed by BGA (results not shown). BGA can be very abundant on narrow leaf lupins late in the season, and it is therefore also possible that plants are more susceptible to aphid feeding during flowering and podding (Ofuya, 1993).

The severe damage by the new BGA has only been demonstrated in glasshouse studies and not yet confirmed in field studies. However, we have collected a second population from near Kybybolite, South Australia with similar virulence on tolerant lucerne (data not presented) and this suggests that the new BGA occurs in a major pasture seed production area of South Australia. BGA only reproduce asexually in Australia (Edwards 2008) which means the virulence of the new BGA will not be diluted with sexual mating with less virulent biotypes of BGA. Given the virulence of the new BGA in our glasshouse studies we are concerned about the threat it poses to pasture and grain legumes in Australia. While we do not know how widespread the new BGA is, we do recommend agronomists and farmers monitor legume crops and pastures. Large BGA populations or unexpected plant damage should be controlled with dimethoate, chlorpyrifos or pirimicarb. Treating lucerne seed with the insecticide imidacloprid provided good protection to lucerne seedlings, and primary producers should use insecticide treated seeds when planting new pastures.

The preliminary results presented in this paper will be explored in greater depth during a RIRDC-funded project to confirm damage and growth rates of BGA populations collected from around Australia on a broad range of species. We aim to develop a molecular based assay for identifying the distribution of this new BGA and assist the pasture and pulse industries in preventing the severe damage that may occur if this aphid becomes widespread in Australia.

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