

## Virulence of Russian Wheat Aphid, *Diuraphis Noxia* (Kurdjumov) (Homoptera: Aphididae) Populations in Kenya

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### Abstract

The Russian wheat aphid (RWA) *Diuraphis noxia* (Kurdjumov) is a serious pest of wheat in Kenya. Development and use of RWA resistant wheat (*Triticum aestivum* L.) varieties, has been constrained by RWA populations evolving with differential virulence to given resistant host plants. To fully exploit host plant resistance (HPR) in management of RWA, local populations of RWA have to be evaluated for differential virulence and biotypes in order to develop and deploy cultivars that exhibit cross biotype resistance. A study was conducted at KARI-Njoro to characterize virulence of RWA populations from the endemic areas (Eldoret, Mau Narok, Njoro and Egerton) in Kenya. A factorial experiment in randomized complete block design replicated three times was set up to evaluate seedling resistance to RWA with variety and aphid collection source as main factors in the screen house. Five adult RWA aphids from each of the four collection locations were used to infest four host genotypes; PI624933-1 containing Dn4 gene, 2414-11-2 containing Dn7 gene, KRWA9 which contains an unknown Dn gene and a susceptible check, K.KWALE, for 28 days to determine virulence of the RWA aphids to seedlings of the four wheat genotypes in the greenhouse. Data was recorded on damage scores, plant height, plant height reduction, shoot biomass and biomass reduction of test plants 28 days after infestation. Results of an analysis of variance of these plant parameters show that Egerton population was more virulent than populations selected from other areas as it caused more damage on resistant lines.

**Keywords:** *Diuraphis noxia*, biotype, virulence, RWA, Wheat.

### Introduction

*Diuraphis noxia* (Kurdjumov) (Homoptera Aphididae) is a new invasive pest of wheat, barley and other small grains in Kenya. Since its discovery in Kenya in the mid 1990's, the aphid has become an important pest of barley, *Hordeum vulgare* L., and wheat, *Triticum aestivum* L. *D. noxia* feeding significantly reduces chlorophyll and carotenoid content in susceptible plants. This chlorophyll reduction has an effect on plant yield (Heng-moss *et al*, 2003). Plant resistance has been considered an especially useful way to control *D. noxia*, because the aphid's habit of feeding within rolled wheat leaves may limit the effectiveness of contact insecticides and some natural enemies (Burd *et al.*, ;Brewer *et al.* 2005) besides, using for aphid management is cheaper and has no negative effects on the environment. The importance of host plant resistance in an integrated pest management program is however limited by development of virulent biotypes. Insect biotypes are infraspecific classifications based on biological rather than morphological characteristics, they are generally morphologically indistinguishable. Insect biotypes have been described as members of an insect species with similar genetic composition for a biological attribute (Saxena and Burrion, 1987) where as Diehl and Bush, (1984) described biotypes based on survival and development on a particular host and by host preference for feeding, oviposition or both. Insect biotypes including *Diuraphis noxia* biotypes have traditionally been described by their ability to damage crops that express host plant resistance. (Puterka *et al.*, 1992). As virulent RWA biotypes with superior fitness replace the previously avirulent RWA biotype populations, the result is breakdown of resistance and ineffective control of RWA. Virulent biotypes of RWA have been reported in the USA (Haley *et al* 2004), South Africa (Tolmay, 2007), Asia (Dolatti *et al*, 2005), Europe, (Basky, 2003) and South America (Smith *et al*, 2004). Genetic studies of Kenyan populations of *D.noxia* demonstrated that there is limited genetic variability among the biotypes under study (Maling'a *et al.*, 2007). Kiplagat, (2005) however found that some Kenyan populations of *D. noxia* were virulent to wheat containing *Dn4* gene for *D noxia* resistance. Malinga, 2007 also found that despite the Kenyan populations showing little genetic variation, the same populations had variations in development and population growth. In this study our primary goal was to characterize virulence of Kenyan biotypes of RWA on selected resistant genotypes of wheat with a view to identify the most appropriate genotypes to incorporate into Kenyan breeding programmes in order to develop a variety of wheat that is resistant to RWA populations in Kenya.

### Materials and Methods

The study was conducted at KARI-Njoro located in the lower highlands (LH<sub>3</sub>), at an altitude of 2166 meters above sea level.

### Plant material

Wheat genotype Kkwale, KRWA9, PI624933-1 and 2414-11-2 2414-11-2 were used. In our earlier experiment Kkwale had been found susceptible and the other three wheat lines were resistant (results not published).

### RWA Populations

RWA populations were collected from the endemic areas (Eldoret, Mau Narok, Njoro and Egerton) in Kenya during 2010. Kenya Pasa (susceptible) wheat, used as the rearing plant, was grown in a sterilized potting mixture composed of forest soil, sand and manure ratio 3:1:1. The potting mixture was amended with 50 kg/ha equivalent of DAP. 10 seeds each of Kenya Pasa (K. Pasa) were planted 2.5 cm deep in the potting mixture in a 1L plastic pot and the pots placed in a water bath in an insect rearing box to keep the emerging seedlings clean from aphid contamination in the greenhouse. When rearing plants reached Zadocks et al. (1974) growth stage 12, a single adult female RWA was settled in the leaf whorl using a fine hair brush. The plants were watered regularly by replenishing water in the water bath after every three days so that the seedlings were not water stressed.

Environmental conditions were  $18 \pm 2^{\circ}\text{C}$  with a photoperiod of 12:12(L:D) h. The aphid was allowed to multiply freely to form a colony. The insect rearing boxes were kept a minimum 10m from each other to eliminate accidental contamination of clones by mixing. The four established clones above were used to test for variation in the ability to cause feeding damage to four wheat genotypes.

### Screening protocol

The experiment was a factorial experiment in randomized complete block design replicated three times. Wheat genotype and *Diuraphis noxia* (origin) collection point were the two factors. Wheat genotype had four levels (Kkwale, KRWA9, PI624933-1 and 2414-11-2 2414-11-2) whereas RWA origin had five levels (Egerton, Eldoret, Mau Narok, Njoro and a non infested control). The experiment was conducted in a screen house during the period September 2010 to January 2011.

Two seeds of the test material were planted in a potting mixture as described earlier in a 1L pot. After emergence, at Zadocks et al. (1974) growth stage 10, they were thinned to leave one seedling per pot. The single seedling was infested with five aphids placed in the leaf whorl using a fine hair brush at growth stage 12 (Zadoks et al., 1974). The infested seedlings were then caged using polyester mesh supported on wires and the aphids left to multiply and feed on the test plants for 28 days. Three seeds were planted per pot and later thinned to two seedlings per pot. Water was supplied regularly by filling the water bath until the soil in the pots was ascertained to be wet by visual examination. Scoring for overall plant damage was done at 7 days, 14 days, 21 days and 28 days post inoculation, the damage to the test plants were qualitatively evaluated using a 1-9 scale (Table, i) where 1-resistant; 9-susceptible (Maling'a, 2007; Tolmay, 1999)

### Traits measured

Plant height was measured 28 days after infestation (DAI). The plants were then cut at the soil surface and weighed to determine fresh weight. The sampled plants were dried at  $105^{\circ}\text{C}$  for 48hrs, and weighed to determine above ground biomass. Proportional height, fresh weight and dry weight was determined using the formulae

$$DWT = \left( \frac{D_c - D_t}{D_c} \right) * 100 \dots \dots \dots \text{Equation 1}$$

- DWT- Proportional reduction
- $D_c$ - Value measured on non infested control plant
- $D_t$ - Value measured on infested plant

. Temperatures range during the duration of the experiment was between 18–28°C

### Statistical analysis

Analysis of variance (ANOVA) was performed on collected data using Genstat (, Significant differences in treatment means were separated using Least significant difference (LSD) at  $\alpha = 0.05$  level of significance.

### Results and Discussion

There were significant differences in damage level on wheat genotypes at different duration of RWA infestation. All wheat genotypes developed damage symptoms associated with RWA infestation as early as 7 days post infestation. (Figure 1).

Wheat genotype varied significantly in plant damage resulting from RWA biotypes on all days when plant damage was assessed. As expected, wheat K. Kwale was generally susceptible throughout the period while

wheat KRWA9 was moderately resistant. Wheat PI624933-1 PI624933-1 and 2414-11-2 were generally moderately resistant to RWA attack (Figure 1).

Aphid biotype caused significant damage on wheat genotypes. The biotypes also varied in their virulence on wheat genotypes (Figure 2). At 7 days post infestation, all aphid biotypes caused plant damage with Egerton, Eldoret and Njoro biotypes causing the most damage. Irrespective of wheat genotype, Mau Narok biotype caused the least damage on infested wheat. Njoro Egerton and Eldoret biotypes emerge as the most virulent biotypes 21 days after infestation and are significantly different from Mau Narok biotype,

There was significant interaction of RWA population and Wheat genotype on four instances when damage score was assessed indicating that populations varied in virulence depending on wheat genotype (Figure 3 and 4). The degree of damage also depended on the duration of infestation for some genotypes. All RWA populations significantly damage wheat but the degree of damage now depends on the RWA population infesting the wheat and the duration under which wheat remains infested.

There were no significant differences among RWA populations on PI624933-1 and 2414-11-2 2414-11-2 at 14, 21 and 28 days of RWA infestation. The two wheat genotypes were moderately resistant to all RWA populations that were tested. Significant differences were noted among populations on KRWA9 during the entire period of RWA infestation. The genotype was clearly susceptible to all populations of RWA after 7 and 14 days of infestation with Njoro and Eldoret populations being the most virulent during this period. Mau narok population was the least virulent to KRWA9 during the entire period of evaluation. KRWA9 has significantly lower mean damage score 21 and 28 days after infestation. There was significant variation in the reduction of seedling growth attributed to *Diuraphis noxia* populations. Percent reduction of plant height varied significantly across wheat genotypes. PI624933-1 and 2414-11-2 2414-11-2 were the tallest plants followed by KKWALE and KRWA9 28 days after infestation with RWA populations (Table ii). There was significant reduction in wheat shoot fresh and dry weight 28 days after infestation of bread wheat with *Diuraphis noxia*. PI624933-1 and 2414-11-2 2414-11-2 had the lowest height, and shoot weight reductions, an indication that these two genotypes are better at tolerating RWA infestation at seedling stage. Differences among individual wheat genotypes were expected and arise from feeding by RWA that causes infested plants to become stunted and genotype characteristics in relation to infestation.

RWA populations varied significantly in their effect on, % leaf reduction, and % height reduction 28 days after infestation. (Table iii). This is an indication that all RWA populations cause plant stunting when they infest wheat plants, however the percent reduction differs significantly among populations.

Egerton population caused the highest % reduction in plant height followed by Eldoret and Njoro population respectively. Mau Narok population caused the least reduction in plant height among RWA populations.

There were significant differences in % reduction in shoot fresh weight of test plants, (Table iii) Test plants infested with Egerton population had the least shoot fresh weight and the population had the highest % fresh shoot weight reduction among RWA populations.

There were significant two way interactions between host genotype and aphid population in some growth measurements of wheat seedlings. Significant interaction was observed in plant height reduction on wheat genotype Krwa9 (Table IV). Significant interaction was also observed in % reduction in plant height, % reduction in shoot fresh weight, and % reduction in shoot dry weight on KKWale, PI624933-1 PI624933-1, and 2414-11-2 2414-11-2, (Tables iv and v).

Table, viii shows Pearson's correlation coefficients among parameters of wheat infested with RWA and RWA damage scores at 21, and 28 days in the greenhouse. Significant positive associations were observed between damage score and plant height reduction, percent shoot fresh weight reduction and shoot dry weight reduction. Allowing RWA to infest wheat results in stunted growth and reduced straw weight. A significant negative relationship exists between damage scores and plant height, shoot fresh weight and shoot dry weight.

## Discussion

Symptoms of Russian wheat aphid damage started manifesting in all wheat genotypes 7 days after infestation. Maling'a, (2007) also found that symptoms of damage started to manifest in both resistant and susceptible plant entries as early as seven days after first infestation, thus for effective management, control of the Russian wheat aphid should start early to minimize yield losses due to aphid damage. Though all populations caused damage on wheat genotypes, some populations caused significantly more damage. Njoro populations caused the severest damage symptoms though mau narok biotype could cause severe damage symptoms only on 2414-11-2 2414-11-2 indicating a possible resistance breaking variant of RWA in Kenya. This findings concur with Kiplagat, (2005) who found that Nakuru RWA populations were more virulent compared to RWA populations from other wheat growing regions. All four Russian wheat aphid populations established from single female aphids collected from the various wheat growing regions in Kenya were virulent to Kenya Kwale which does not have any Dn resistance gene. K.Kwale is a highly popular variety in Kenya grown by almost 60% of wheat farmers

even though it is susceptible to *Diuraphis noxia*. The variety would form a good background in any breeding program to breed wheat resistant to RWA. The other resistant introductions had varying damage ratings depending on RWA population used to infest the resistant line and the duration of infestation. The line RWA9 had high damage values in the first and second week and this could be due to the line having been used for a long time in breeding programs in Kenya to develop RWA resistant wheat and the aphid populations could be acclimatized to the resistance in RWA9. Jyoti and Michaud (2005) reported high damage values for Trego infested with USA RWA biotype 1 because the biotype had been acclimatized on the variety. 20 generations of the aphid had been raised on the variety and were acclimatized to it. This indicates that when a resistant source is exposed to an aphid population for a long time, resistance may begin to break down as the aphid becomes acclimatized on the variety developing novel strategies for neutralizing resistance factors in the variety. This is an indication that a resistant variety cannot be grown indefinitely as a control measure, therefore the process of searching for new sources of resistance should be continuous. Botha et al., (2005) also postulated that several defense strategies could be employed in wheat defenses including systemic acquired resistance. KRWA9 responds to RWA attack by becoming moderately resistant over time, this could be due to the fact that it may take time for the plant to build up defence factors from the first time of aphid infestation when the plant recognizes proteins in the aphid saliva during the initial sampling before settling. It also indicates that the Dn resistance gene in KRWA9 may be responsible for systemic acquired resistance observed in KRWA9. The two resistant lines, PI624933-1 and 2414-11-2 were generally resistant and had low damage ratings for all RWA populations except that PI624933-1 had uncharacteristically high damage score when infested with Mau Narok RWA population. There is a high likelihood of the Mau Narok RWA population having evolved into a virulent resistance breaking biotype and therefore an indication that PI624933-1 would not be a suitable resistant parent in a breeding program to develop RWA resistant wheat in that region. It also could mean that RWA population in Mau Narok will need to be managed using resistant materials that contain more than one resistant gene and therefore have different modes of resistance.

There was also some reductions in some of the growth parameters measured such as plant height due to RWA infestation. Plant damage due to *Diuraphis noxia* is associated with developmental, morphological, physiological and biochemical processes in the host plant. Reduction in biomass of wheat plants infested with *Diuraphis noxia* was also reported by Ni and Quinsenberry, (2006). The greater the reduction, the more virulent the population.

Russian wheat aphid populations in Kenya have been evaluated for biotype development (Malinga et al., 2007). However this study has clearly indicated that there are at least two possible biotypes based on the result of the study.

### **Conclusion**

The Russian wheat aphid populations tested showed distinct differences in how they affected damage and growth of resistant and susceptible wheat entries. Njoro and Egerton populations emerge as the most virulent populations whereas Mau Narok population emerges as the least virulent of all populations tested. This shows that there are at least two distinct RWA biotypes in Kenya. Among the wheat genotypes tested, PI624933-1 and 2414-11-2 performed more uniformly in plant damage scores, had the least reduction in height, shoot fresh weight and shoot dry weight reduction and generally exhibited moderate resistance to the RWA populations tested.

### **Recommendations**

Tolerance to RWA should be incorporated into breeding programs in order to slow down biotype development by reducing selection pressure. Symptoms of RWA damage are manifested as early as seven days after infestation. Therefore, management of the pest should start as soon as RWA is seen in the field.

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- Tables and figures

## Tables and figures

Table I. Effect of wheat genotype on growth of selected bread wheat seedlings infested with *Diuraphis noxia* populations for 28 days.

HOST GENOTYPE	% PLANT HEIGHT REDUCTION	% FRESH SHOOT WEIGHT REDUCTION	% DRY SHOOT WEIGHT REDUCTION
KRWA9	36.3	40.76	11.72
KWALE	25.3	25.85	7.43
PI 624933	6.8	17.40	4.38
2414-11-2	7.2	15.67	6.36
LSD	1.8	2.2	2.2
%CV	12.7	12.2	41.0

Table II. . Effect of rwa (*Diuraphis noxia*) populations on growth of bread wheat seedlings after for 28 days of infestation

<b>RWA POPULATION</b>	<b>% Plant height reduction</b>	<b>%Fresh shoot weight reduction</b>	<b>%Dry shoot weight reduction</b>
UNINFESTED CONTROL	0.0	0.0	0.0
EGERTON	26.9	36.4	10.0
ELDORET	24.6	29.6	10.2
MAU NAROK	20.1	28.5	8.3
NJORO	22.9	30.1	8.9
LSD	2.0	2.5	2.5
CV	12.7	12.2	41.0

Table III. Effect of rwa (*Diuraphis noxia*) population on krwa9 and 2414-11-2 wheat genotype seedling height, shoot fresh weight and dry weight reduction 28 days after infestation

<b>RWA Population</b>	<b>KRWA9</b>			<b>2414-11-2</b>		
	<b>% Plant height reduction</b>	<b>%Fresh shoot weight reduction</b>	<b>% Dry shoot weight reduction</b>	<b>% Plant height reduction</b>	<b>%Fresh shoot weight reduction</b>	<b>% Dry shoot weight reduction</b>
UNINFESTED	0.0	0.0	0.0	0.0	0.0	0.0
EGERTON	53.3	52.3	15.0	15.8	25.6	7.5
ELDORET	40.9	50.4	14.8	7.1	13.4	9.5
MAU NAROK	47.1	50.8	14.7	5.2	25.3	7.5
NJORO	40.1	50.2	14.1	7.9	14.0	7.4
LSD	4.9	3.1	4.9	4.5	4.7	4.7
CV	7.2	4.1	22.3	32.9	15.8	38.8

Table IV. Effect of RWA (*Diuraphis noxia*) population on kwale and PI624933-1 wheat genotype seedling height, shoot fresh weight and dry weight reduction 28 days after infestation

<b>RWA Population</b>	<b>KWALE</b>			<b>PI624933-1</b>		
	<b>% Plant height reduction</b>	<b>%Fresh shoot weight reduction</b>	<b>% Dry shoot weight reduction</b>	<b>% Plant height reduction</b>	<b>%Fresh shoot weight reduction</b>	<b>% Dry shoot weight reduction</b>
UNINFESTED	0.0	0.0	0.0	0.0	0.0	0.0
EGERTON	29.1	36.2	9.8	9.5	31.5	7.8
ELDORET	42.6	32.6	11.2	7.7	21.9	5.3
MAU NAROK	23.1	23.5	6.4	4.9	14.5	4.6
NJORO	31.4	36.9	9.7	12.0	19.2	4.2
LSD	4.5	4.8	4.8	3.2	2.9	3.5
CV	9.5	34.2	34.2	24.7	8.8	42.6

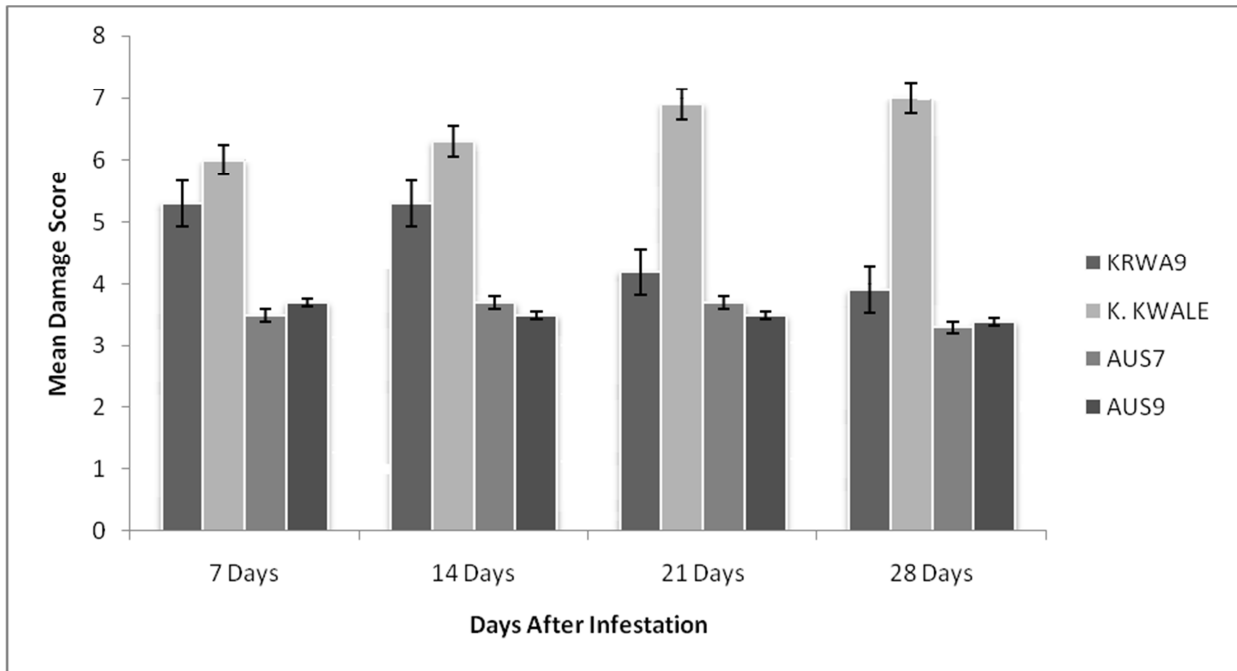


Figure 1. Mean plant damage values on wheat at various days after infestation with *Diuraphis noxia*.

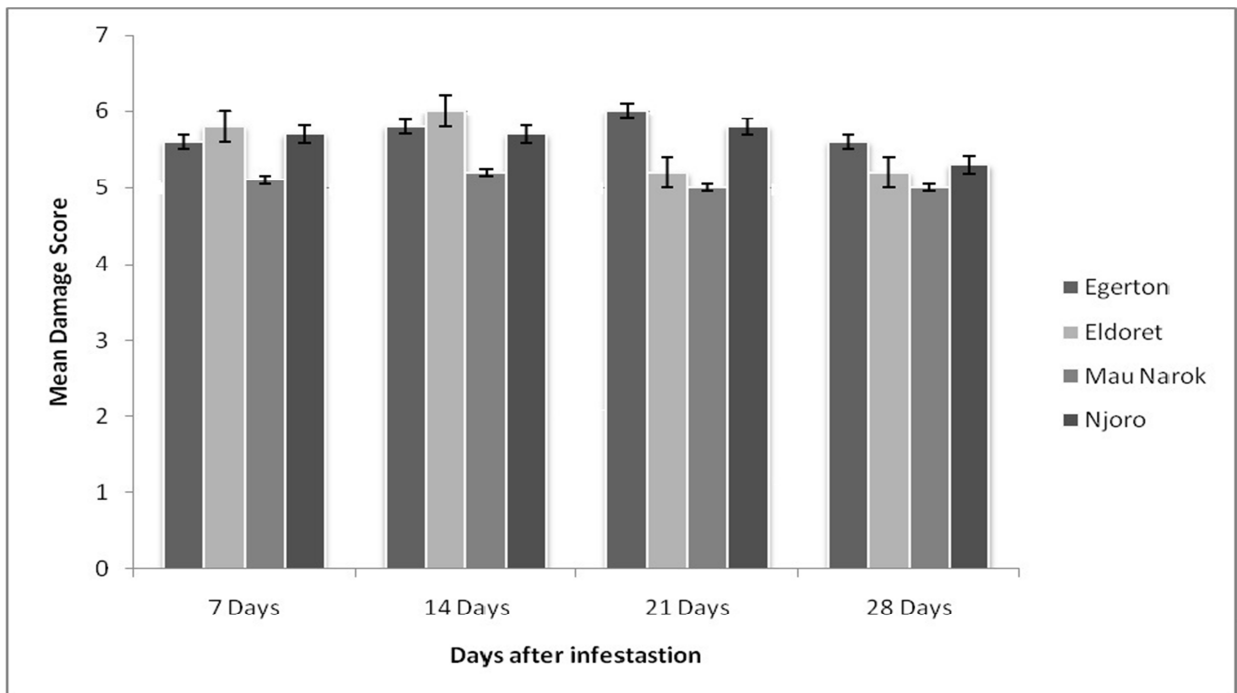


Figure 2. Mean plant damage score at varying days of wheat infestation with *Diuraphis noxia* biotypes.

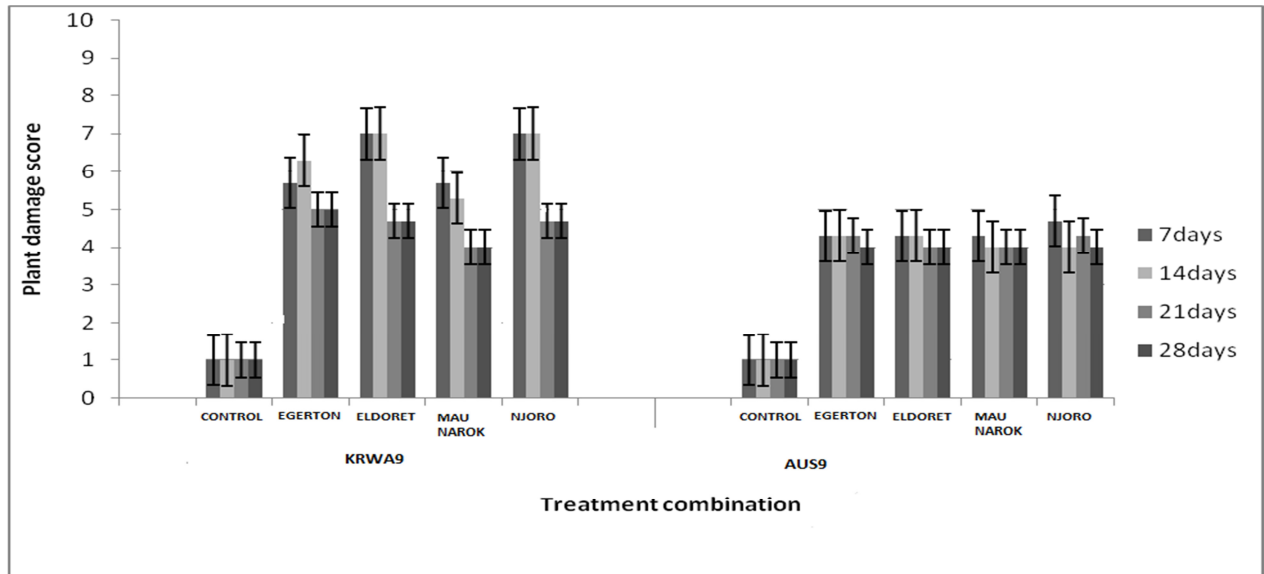


Figure 3 Level of plant damage caused by different aphid (*Diuraphis noxia*) populations on KRWA9 and 2414-11-2 2414-11-2 wheat genotypes at varying times of infestation

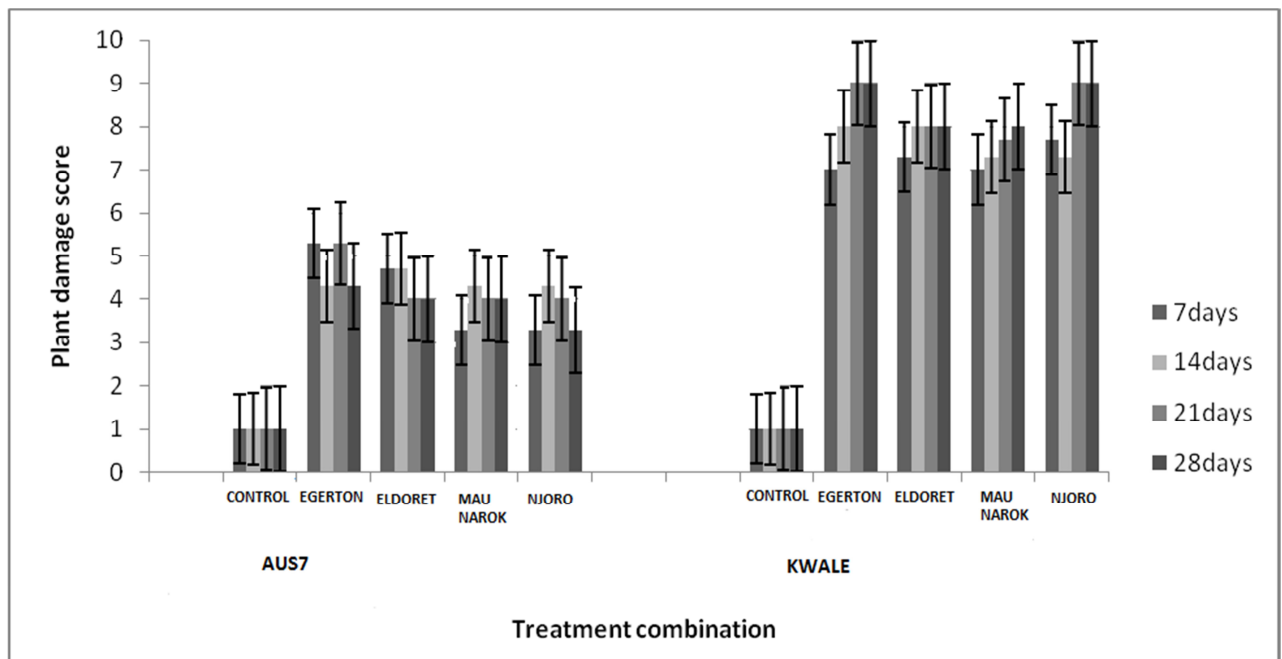


Figure 4. Level of Plant damage caused by different *Diuraphis noxia* aphid populations on PI624933-1 and Kwale wheat genotype at varying times of infestation



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