Chapter 4

Development of an artificial diet for the Russian wheat aphid (*Diuraphis noxia*)

4.1 Abstract

The Russian wheat aphid, *Diuraphis noxia* (Mordvilko), has been a serious insect pest of wheat (*Triticum aestivum*) since its detection in South Africa during 1978. Insect damage has been curbed by the use of resistant wheat cultivars and chemical control, but little information is available on the exact mechanism of resistance that these cultivars possess. The development of an artificial diet would enable researchers to test possible resistant compounds for their deterrency to the Russian wheat aphid. Three sucrose concentrations (10, 20 and 30%) were tested to determine the optimal concentration required by the aphid. The aphid was found to prefer an optimal sucrose concentration of 20%. Further results showed that the Russian wheat aphid had dietary requirements for three essential amino acids (methionine, leucine and tryptophan). The addition of these amino acids to 20% sucrose resulted in an increase in the reproductive rate of the aphid. The addition of potassium phosphate and magnesium chloride was also found to be beneficial to the Russian wheat aphid. These two salts increased the life span of the aphid. In this study, an artificial diet was developed for the Russian wheat aphid that is simple, yet effective.

4.2 Introduction

The Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae), is a serious insect pest of wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*). It was first discovered in South Africa during 1978 on wheat in the northern Free State and has since become the most destructive pest on wheat in this country.

Feeding damage by the RWA results in chlorotic lesions on the leaves (Walters *et al.*, 1980) and a reduction in growth and development of susceptible plants (Burd & Burton, 1992). The aphids occur mainly on the new growth of plants, in the axils of the leaves or within curled leaves. RWA feeding on wheat causes a reduction in leaf area due to a combination of leaf stunting and new leaves not unfolding (Burd & Burton, 1992).

During nutritional requirement investigations of aphids in general, several chemically defined artificial diets have been developed. These artificial diets have been used as a feeding system in elucidating the nutritional requirements of aphids (Mittler, 1967), for studying their salivary proteins (Urbanska *et al.*, 1998; Cherquil & Tjallingii, 2000), as well as testing the deterrency of certain potentially resistant compounds (*e.g.* plant proteinase inhibitors) to aphids (Tran *et al.*, 1997).

Very little has been published on an artificial diet for the RWA. Damsteegt *et al.* (1992) exposed the RWA for 24 hours to a simple 20% sucrose solution containing *Rhopalosiphum padi* viruses (RhPV) to determine if the aphid transmitted viruses. Tran *et al.* (1997) reported on a more

complex diet than that of Damsteegt *et al.* (1992). However, this complex diet was developed specifically for the corn leaf aphid (*Rhopalosiphum maidis*) and was less useful for other cereal aphids (Kieckhefer & Derr, 1967). The RWA was only kept on this diet for 3 days to test the effectiveness of plant proteinase inhibitors in controlling the aphid on wheat. Thus, a simple yet effective artificial diet still needs to be developed for the RWA.

Investigations into the use of artificial diets for other aphids revealed that several constituents might be included in an artificial diet for the RWA. Methionine is strongly phagostimulatory to *Myzus persicae* (Mittler & Dadd, 1964), but not for *Aphis fabae* (Leckstein & Llewellyn, 1974); although methionine is essential for *A. fabae* to reach adulthood and to synthesize proteins. Furthermore, the RWA has endosymbiotic bacteria (*Buchnera* sp.) which overproduce limiting amino acids which benefit their hosts (Douglas, 1998). The bacteria synthesize two essential amino acids for the RWA, namely tryptophan (Lai *et al.*, 1996) and leucine (Thao *et al.*, 1998). There is a reduction in the number of gene copies of these two amino acids carried by the endosymbiotic bacteria in the RWA (Lai *et al.*, 1994; Thao *et al.*, 1998). Telang *et al.* (1999) found that the concentrations of both leucine and tryptophan increased significantly in infested susceptible wheat. Therefore a more efficient mechanism for utilizing nitrogen by the RWA has resulted in loss of copies of these genes by *Buchnera*. Conversely, increased levels of tryptophan and leucine in infested susceptible wheat may have reduced the need for provisioning by the endosymbiont. Retention of single copies of the genes has been selected for when the aphid encounters nutritionally deficient hosts. Methionine, tryptophan and leucine were therefore included in the artificial diet.

Omission of potassium phosphate and magnesium chloride from the diet of *M. persicae* resulted in a reduction in longevity and reproduction of these aphids (Dadd & Mittler, 1965). Similarly, survival and growth of the pea aphid, *Acyrthosiphon pisum*, was significantly increased by the presence of these two major salts (Auclair, 1965). Hence, potassium phosphate and magnesium chloride were included in the artificial diet.

The aim of this study was to develop an artificial diet for the RWA. An effective, simple artificial diet would enable RWA deterrence studies to be done to identify potential resistant compounds. These would be included in an artificial diet to determine their effectiveness in inhibiting aphid fecundity and longevity.

4.3. Materials and Methods

4.3.1 Aphids

Wheat (*Triticum aestivum* cv. 'Palmiet') was grown in a greenhouse at a temperature of 25±1°C. This cultivar is susceptible to the RWA. RWA obtained from the Agricultural Research Council – Small Grain Institute (ARC-SGI) in Bethlehem, South Africa, was maintained on this wheat.

4.3.2 Artificial diet

4.3.2.1 Testing optimal sucrose concentrations

Adult apterous aphids were removed from RWA colonies and placed on wheat leaves cut from the 'Palmiet' cultivar. Three adults were placed on each leaf. These leaves were placed individually in a petri dish (Michels *et al.*, 1987). The cut ends were placed between moistened paper towels to prevent desiccation of leaves. The leaves were replaced every three to four days (Michels & Behle, 1989), or when they showed signs of desiccation. The petri dishes were maintained at 25±1°C with a photoperiod of 12:12 (L:D). A fine horse-hair paint brush was used to transfer aphids to ensure that they were not harmed (Aalbersberg *et al.*, 1987).

Nymphs produced by the adults were removed daily and placed individually on a wheat leaf as described above. The age of these nymphs (first instar) was therefore known. Individual nymphs were observed daily for moulting and survival. The presence of exuviae was used to determine that the aphid had moulted (Aalbersberg *et al.*, 1987). This indicated that the aphid had entered the next instar. When the aphids reached the adult stage (fifth instar), each adult was individually

placed in a glass test-tube (135 mm in length), similar to that described by Mittler and Dadd (1964).

The open end of the test-tube was covered with a stretched Parafilm®M sachet containing 100 µl artificial diet (Kieckhefer & Derr, 1967). The first membrane was stretched to a tenth of its original thickness and placed over the top of the test-tube. The diet was then placed on the taut surface and covered with a second stretched membrane. Covering the sachet was a piece of yellow cellophane (Kieckhefer *et al.*, 1976; Tran *et al.*, 1997), which acted as an attractant to the aphid. The test-tube was placed upside down and gently tapped till the aphid fell onto the Parafilm®M sachet. The test-tubes were maintained at 25±1°C with a 12:12 (L:D) photoperiod.

Three diets were tested: a 10% sucrose, 20% sucrose, and 30% sucrose solution. These diet solutions were buffered to pH 7.0 with a 100 mM K_2HPO_4 solution (Bouwer, pers. comm[a].). The diets were filter sterilized (0.45 µm pore size) and stored at 4°C. Each adult/diet combination was replicated 20 times. Sachets were replaced every 24 hours to prevent bacterial contamination (Kieckhefer & Derr, 1967). Adults were observed daily for survival and production of nymphs.

4.3.2.2 Testing diet A and B

Adult aphids were placed on one of two artificial diets (Table 4.1). The optimal sucrose concentration used in Diet A and B was as determined in the previous section. Diet A contained all the components indicated in the table and Diet B was similar to Diet A except for the

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exclusion of magnesium chloride and potassium phosphate.

Newborn nymphs were placed on 'Palmiet' wheat leaves as discussed above. The adults that developed from these nymphs were placed on either Diet A or B. Twenty replicates were done on each diet. Adults were observed daily for survival and the production of nymphs.

4.3.3 Statistical analyses

Results were analyzed and compared for progeny production and survival of aphids when placed on the five artificial diets. Data was subjected to a Student's t-Test (P = 0.05; Freund, 1976) using the Systat® 7.0.1 (1997) software.

Table 4.1. Composition of Diet A and Diet B.

Components	A[a]		B[a]
L-amino acids			
Methionine	0.10g	Akey & Beck, 1971	0.10g
Leucine	0.20g		0.20g
Tryptophane	0.10g		0.10g
Sucrose	20.00g		20.00g
$MgCl_2.6H_2O$	0.20g	Dadd & Mittler, 1965	--
K_3PO_4	0.25g		--

[a]pH adjusted to 7.0 with 100 mM K_2HPO_4, and ddH_2O added to make 100 ml of diet.

4.4. Results

RWA adults were placed on diets of differing sucrose concentrations to determine the optimal sucrose concentration for the aphid. Three sucrose concentrations were tested, namely, 10%, 20% and 30%. The optimal sucrose concentration was then included in testing two further diets, Diet A and Diet B.

Testing optimal sucrose concentrations. RWA adults placed on the three sucrose diets (10%, 20% and 30% sucrose) showed that those on the 20% and 30% sucrose diets were more likely to overcome the adjustment from feeding on wheat leaves to feeding on the artificial diet and to become reproductively successful (Table 4.2). Only 45% of adults became reproductively successful on the 10% sucrose diet, as compared to 85% and 70% on the 20% and 30% sucrose diets, respectively.

Aphids that were reproductively successful on the three sucrose diets are presented in Table 4.3. Significantly more nymphs per adult were produced on the 20% than 10% sucrose diets. Aphids produced a comparative count of 5.82 nymphs on the 20% sucrose diet versus 2.56 nymphs on the 10% sucrose diet. Also, significantly more nymphs per day were produced on the 20% sucrose compared to the 30% sucrose diet (0.40 and 0.23 nymphs per day, respectively).

Table 4.2. Russian wheat aphid adults surviving to become reproductive. Twenty adults were placed on each of the five diets at 25±1°C and a 12:12 (L:D) photoperiod.

Diet	Adults survival and reproductive success
10% sucrose	45%
20% sucrose	85%
30% sucrose	70%
A	90%
B	80%

Table 4.3. Average number of nymphs produced per Russian wheat aphid, average number of nymphs produced per day and average life span on each of the five diets at 25±1°C at a 12:12 (L:D) photoperiod.

Diet	Average nymphs/ adult ± SD[y,z]	Average nymphs/ day ± SD[y,z]	Average life span (days) ± SD[y]
10% sucrose	2.56±2.01[a]	0.22±0.17[ab]	11.35±1.66[a]
20% sucrose	5.82±2.38[b]	0.40±0.19[a]	21.80±7.55[b]
30% sucrose	4.29±1.73[ab]	0.23±0.10[b]	20.00±7.75[b]
A	6.44±4.73[ab]	0.62±0.28[c]	19.60±9.53[b]
B	5.75±4.01[b]	0.78±0.35[c]	14.60±4.70[c]

[y] Means in the same column followed by the same letter are not significantly different (P>0.05) based on the Student's t-Test (Freund, 1976).

[z] The first and second columns only includes adults that survived to become reproductively active.

Longevity of the aphids on the three diets is presented in Table 4.3. The aphids had a significantly longer life span on the 20% and 30% sucrose diets compared to those on the 10% sucrose diet. Aphids had a longevity of 11.35 days on the 10% sucrose diet which was significantly less than the 21.80 and 20.00 days on the 20% and 30% sucrose diets, respectively.

Testing Diet A and B. The more complex Diets A and B showed similar results to the 20% and 30% sucrose diets when reproductive activity was compared (Table 4.2). Ninety percent of adults on Diet A successfully overcame the change from feeding on wheat leaves to an artificial diet as compared to that of diet B (80%).

RWA reproduction on Diet A and B is presented in Table 4.3. No significant differences were found for the number of nymphs per adult or number of nymphs per day on either of Diet A or B. However, significantly more nymphs per day were produced on Diets A and B compared to that of the three sucrose diets. The addition of three essential amino acids, methionine, leucine and tryptophan, showed a significant increase in the number of nymphs being produced each day.

RWA longevity studies showed that aphids survived significantly longer on Diet A than on Diet B (19.60 and 14.60 days, respectively) (Table 4.3). The omission of the two major salts in Diet B resulted in a significantly lower longevity when compared to Diet A.

4.5. Discussion

During the development of an artificial diet for the RWA (Diet A), it was found that aphids had a requirement for three important dietary sources. Firstly, a carbon source (sucrose) with an optimum concentration of 20%, that allowed a greater number of aphids to survive and become reproductively active when transferred to the artificial diets. This is probably because sucrose is a feeding stimulant (Dreyer & Campbell, 1987). The 20% sucrose concentration is in agreement with several other studies on aphids. *Myzus persicae* has an optimal sucrose range of 10% to 20% (Mittler & Dadd, 1963), while *Aphis gossypii* has an optimal sucrose concentration of 20% (Turner, 1971). However, not all aphids have similar sucrose requirements. *Acyrthosiphon pisum* required 35% sucrose to survive (Akey & Beck, 1971).

Secondly, the RWA showed a requirement for a nitrogen source (methionine, leucine and tryptophan). The addition of these three essential amino acids to 20% sucrose (Diet B) showed a significant increase in the average number of nymphs being produced each day as compared to that of the 20% sucrose solution. Thirdly, the addition of the two salts (Diet A) resulted in an increased life span. Similar results were found with *M. persicae* and *A. pisum* (Auclair, 1965; Dadd & Mittler, 1965).

Comparison of the life span of the RWA on wheat leaves to that on artificial diets showed similar results to that obtained on Diet A (Michels & Behle, 1988; Bahlmann, unpubl.). However, a reduction in the reproductive rate of the RWA on the experimental artificial diets was found. This could be due to a number of factors. The RWA feeds via a stylet that follows an

intercellular path until the phloem is reached. Feeding by the RWA results in ultrastructural damage with the chloroplast membrane being degraded. The subsequent release of nutrients from the chloroplast has been hypothesized to benefit the aphid as RWA feeding was shown to enhance the nutritional quality of the phloem (Fouché *et al.*, 1984; Telang *et al.*, 1999; Sandström *et al.*, 2000). The RWA also has an endosymbiont (*Buchnera*) that showed a reduction in the number of gene copies of leucine and tryptophan (Lai *et al.*, 1994; Thao *et al.*, 1998), the same amino acids that showed an increase in infested susceptible wheat (Telang *et al.*, 1999). The diet formulated in this study cannot match the natural phloem sap encountered by the aphid as well as the conditions encountered when the aphid is feeding on a susceptible wheat plant. The plant-insect-endosymbiont interaction therefore plays a complex role in the nutrition of the RWA.

In this study, a simple and effective diet was developed (Diet A) for the RWA. Although, it does not mimic the conditions encountered by the aphid in a wheat plant, it provides an easy and effective method of testing for the efficacy of potentially resistant compounds to the RWA.

4.6. References

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