Problem. Past research on the transmission of the nudivirus OrV in populations of the coconut rhinoceros beetle, *Oryctes rhinoceros*, have shown that adult beetles can become infected in a mixture of sawdust and a slurry of virus-killed larvae or together with virus-infected adults. Field studies have also shown that mated females are more frequently infected than unmated females. Furthermore, larvae either hatching from surface contaminated eggs showed no infection or larvae hatching from eggs laid by virus-infected females showed little or no infection [Zelazny, B. 1976. J. Invertebr. Path. 27(2):221-227].

Experimental design. As depicted in the diagram below the the first set of experimental treatments will consists of 15 mason jars each half-filled with sphagum material containing a male and female. In the first treatment set each of eleven jars will have an infected male and an uninfected female. The second treatment set each of eleven jars will have an uninfected male and infected female. The third treatment acting as a control will have 11 jars each with an uninfected male and female. Male and female beetles will be selected from the laboratory colony and each randomly assigned to a jar. This study will be repeated in three blocks for a total of 15 replicates per treatment. Beetle weights will be taken at the beginning, during (if dead) and end of the 4 week study. Beetles to be laboratory infected will be per os treated with ca. 30 µl V23B virus preparation of unknown concentration (AgResearch, New Zealand). A fresh piece of banana will be added to each jar weekly (removing any old banana pieces). Beetles will be monitored on alternate days for mortality or symptoms of leathergy (i.e. by forcing the beetle to the top of the sphagum to determine if they are unable to right themselves and show active reburrowing into the bedding material). At time of death and at study termination, beetles will be dissected, the internal morphology of the midgut and their general appearance photo-documented, and the midgut removed for viral DNA extraction and polymerase chain reaction (PCR) analysis to determine, if any, virus presence.

Treatment 1 - **I♂U♀**

T-1 I♂U♀ T-2 I♂U♀ T-3 I♂U♀ T-4 I♂U♀ T-5 I♂U♀ — Block 1

T-6 I♂U♀ T-7 I♂U♀ T-8 I♂U♀ T-9 I♂U♀ T-10 I♂U♀ — Block 2

T-11 I♂U♀ T-12 I♂U♀ T-13 I♂U♀ T-14 I♂U♀ T-15 I♂U♀ — Block 3

Treatment 2 - **U♂I♀**

T-1 U♂I♀ T-2 U♂I♀ T-3 U♂I♀ T-4 U♂I♀ T-5 U♂I♀ — Block 1

T-6 U♂I♀ T-7 U♂I♀ T-8 U♂I♀ T-9 U♂I♀ T-10 U♂I♀ — Block 2

T-11 U♂I♀ T-12 U♂I♀ T-13 U♂I♀ T-14 U♂I♀ T-15 U♂I♀ — Block 3

Treatment 3 **Control**

C1 U♂U♀ C2 U♂U♀ C3 U♂U♀ C4 U♂U♀ C5 U♂U♀ — Block 1

C6 U♂U♀ C7 U♂U♀ C8 U♂U♀ C9 U♂U♀ C10 U♂U♀ — Block 2

C11 U♂U♀ C12 U♂U♀ C13 U♂U♀ C14 U♂U♀ C15 U♂U♀ — Block 3

	Date (dead)							
Control			IF/UM			IM/UF		
C1	1/16/2020 ♂ 1/29/2020 ♀		T-1			T-1	1/2/2020 ♂	
C2	1/29/2020 ♂		T-2	2/3/2020 ♂		T-2	1/27/2020 ♀	
C3	1/19/2020 ♂ 1/29/2020 ♀		T-3	1/14/2020 ♂ 2/6/2020 ♀		T-3	1/19/2020 ♂ 2/6/2020 ♀	
C4	1/21/2020 ♂ 1/31/2020 ♀		T-4	1/27/2020 ♀		T-4	1/31/2020 ♂	
C5	1/29/2020 ♂		T-5	1/16/2020 ♂ 1/27/2020 ♀		T-5	1/19/2020 ♀	
C6	1/16/2020 ♀		T-6	1/16/2020 ♀ 1/21/2020 ♂		T-6	1/25/2020 ♀ 1/29/2020 ♂	
C7	1/2/2020 ♂♀		T-7	12/18/2019 ♂ 1/27/2020 ♀		T-7	1/29/2020 ♀	
C8	1/232020 ♂ 1/23/2020 ♀		T-8			T-8	1/19/2020 ♂ 2/6/2020 ♀	
C9	1/25/2020 ♂		T-9			T-9	1/8/2020 ♀	
C10	1/19/2020 ♀		T-10	1/19/2020 ♀		T-10	1/8/2020 ♂ 1/14/2020 ♀	
C11	1/25/2020 ♂		T-11			T-11	1/14/2020 ♂ 1/29/2020 ♀	
C12	1/21/2020 ♀		T-12			T-12	1/19/2020 ♂	
C13			T-13	1/8/2020 ♀ 2/3/2020 ♂		T-13	1/29/2020 ♂ 2/3/2020 ♀	
C14			T-14			T-14	1/14/2020 ♀ 1/29/2020 ♂	
C15			T-15	1/29/2020 ♂ 1/29/2020 ♀		T-15	mating, alive 1/10/2020 1/19/2020♀	
			C21-F/M			C16-M/F	1/14/2020 ♀ 2/3/2020 ♂	
			C22-F/M	2/3/2020 ♀		C17-M/F	1/21/2020 ♀ 1/27/2020 ♂	
			C23-F/M	1/10/2020 ♀ 1/21/2020 ♂		C18-M/F	1/23/2020 ♂ 2/6/2020 ♀	
			C24-F/M	1/23/2020 ♀		C19-M/F	1/29/2020 ♀ 1/31/2020 ♂	
			C-25-F/M			C20-M/F	2/6/2020 ♂	
			C26-F/M			C31-M/F		
			C27-F/M			C32-M/F	1/19/2020 ♂	
			C28-F/M	1/29/2020 ♂		C33-M/F	2/3/2020 ♂	
			C29-F/M	1/10/2020 ♀ 2/6/2020 ♂		C34-M/F	1/27/2020 ♀	
			C30-F/M			C35-M/F		
			C36-F/M	1/10/2020 ♀		C41-M/F	1/27/2020 ♀	
			C37-F/M	1/31/2020 ♂		C42-M/F	1/31/2020 ♀	
			C38-F/M			C43-M/F		
			C39-F/M			C44-M/F		
			C40-F/M			C45-M/F	2/6/2020♀	

bacteria; possible virus


T-1 IM/UF male 1/02


C-7 male 1/2/20


C-7 female 1/2/20


T-7 IF/UM 12/29/19

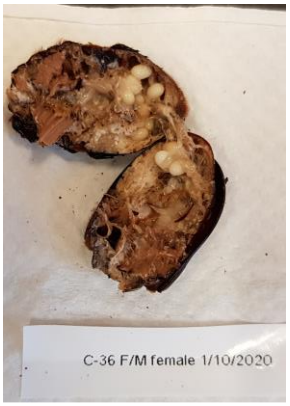
♂


T-13 IF/UM 1/8/2020 female


T-10 IM/UF 1/8/2020 male

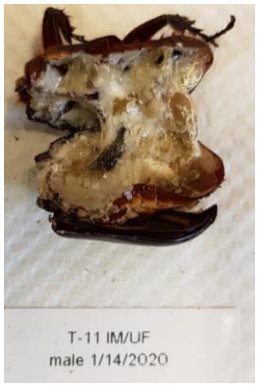

T-9 IM/UF 1/8/2020 female


C-29 F/M female 1/10/202

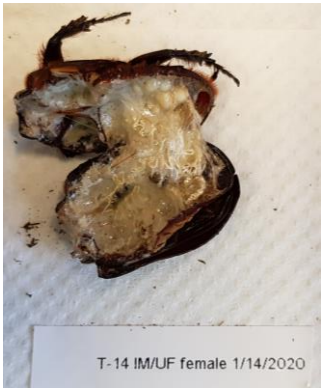

C-36 F/M female 1/10/2020


C-23 F/M female 1/10/2020


T-10 IM/UF
female 1/14/2020


T-11 IM/UF
male 1/14/2020


T-3 IF/UM male 1/14/2020


T-14 IM/UF female 1/14/2020


C-16 M/F female 1/14/2020


T-6 IF/UM female 1/16/202


T-5 IF/UM male 1/16/2020


C-6 female 1/16/2020


C-1 male 1/16/2020


T-5 IM/UF
female 1/19/2020


T-10 IF/UM
female 1/19/2020


T-3 IM/UF male 1/19/2020


T-12 IM/UF male 1/19/2020


C-10 female 1/19/2020


C-3 male 1/19/2020


C-32 M/F
male 1/19/2020


T-15 IM/UF
female 1/19/2020


T-8 IM/UF male 1/19/20


C-17 M/F female 1/21/2020


C-12 female 1/21/2020

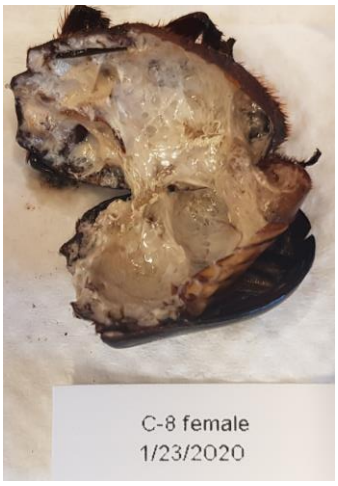

C-4 male 1/21/2020


T-6 IF/UM
male 1/21/2020


C-23 F/M
male 1/21/2020


C-24 female
1/23/2020


C-18 male
1/23/2020

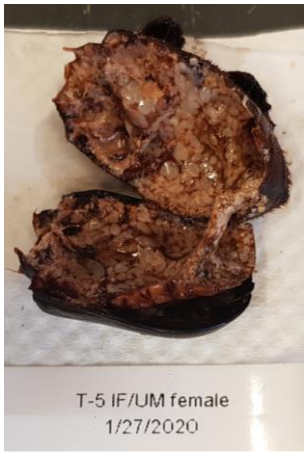

C-8 female
1/23/2020

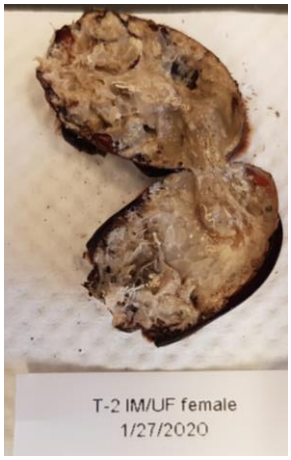

C-8 male
1/23/2020


C-9 female
1/25/2020


C-11 male
1/25/2020


T-6 IM/UF female
1/25/2020


T-5 IF/UM female
1/27/2020


T-2 IM/UF female
1/27/2020


T-4 IF/UM female
1/27/2020


C-34 IM/UF female
1/27/2020


T-7 IF/UM female
1/27/2020


C-17 IM/UF male
1/27/2020


C-41 IM/UF female
1/27/2020


unknown male
1/29/2020


C-3 female
1/29/2020

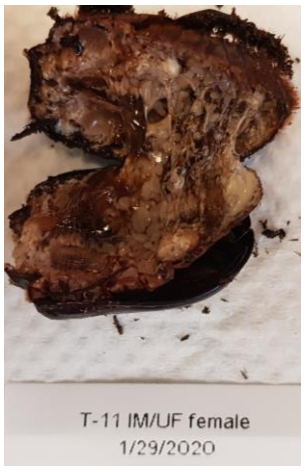

T-7 IM/UF female
1/29/2020


T-6 IM/UF male
1/29/2020


T-14 IM/UF male
1/29/2020


T-15 IF/UM male
1/29/2020


T-15 IF/UM female
1/29/2020


T-11 IM/UF female
1/29/2020


T-13 IM/UF male
1/29/2020


C-1 female
1/29/2020


C-2 male
1/29/2020


C-28 male
1/29/2020


C-19 female
1/29/2020


C-5 male 1/29/2020


T-4 IM/UF
male 1/30/2020


C-42 M/F
female 1/30/2020


C-19 M/F
male 1/30/2020


C-37 F/M
male 1/30/2020


C-4 female 1/30/2020


T-13 IM/UF male
2/3/2020


T-2 IF/UM male
2/3/2020


T-13 IM/UF female
2/3/2020


C-16 M/F male
2/3/2020


C-22 F/M female
2/3/2020


C-33 M/F male
2/3/2020


T-8 IM/UF
male 2/6/2020


C-29 F/M
male 2/6/2020


T-3 IM/UF
female 2/6/2020


T-3 IF/UM
female 2/6/2020

	(CRBs that survived the end of the study- 2/6/2020)							
Control			IF/UM			IM/UF		
C1			T-1	♀♂		T-1	♀	
C2			T-2	♀		T-2		
C3			T-3			T-3		
C4			T-4	♂		T-4	♀	
C5	♀		T-5			T-5	♂	
C6	♂		T-6			T-6		
C7			T-7			T-7	♂	
C8			T-8	♀♂		T-8	♂	
C9			T-9	♀♂		T-9		
C10			T-10	♂		T-10		
C11	♀		T-11	♀♂		T-11		
C12	♂		T-12	♀♂		T-12	♀	
C13	♀♂		T-13			T-13		
C14	♀♂		T-14	♀♂		T-14		
C15	♀♂		T-15			T-15		
Total survived	**10**		Total survived	**15**		Total survived	**6**	
			C21-F/M	♀♂		C16-M/F		
			C22-F/M	♀		C17-M/F		
			C23-F/M	♂		C18-M/F		
			C24-F/M			C19-M/F		
			C-25-F/M	♀♂		C20-M/F	♀	
			C26-F/M	♀♂		C31-M/F	♀♂	
			C27-F/M	♀♂		C32-M/F	♀	
			C28-F/M	♀		C33-M/F	♀	
			C29-F/M			C34-M/F	♂	
			C30-F/M	♀♂		C35-M/F	♀♂	
			C36-F/M	♂		C41-M/F	♂	
			C37-F/M	♀		C42-M/F	♂	
			C38-F/M	♀♂		C43-M/F	♀♂	
			C39-F/M	♀♂		C44-M/F	♀♂	
			C40-F/M	♀♂		C45-M/F	♂	
			Total survived	**21**		Total survived	**15**	


C-25 F/M male
2/10/2020


C-2 female
2/10/2020


C-14 female
2/10/2020


C-24 F/M male
2/10/2020


C-6 male
2/10/2020


C-11 female
2/10/2020


C-13 male
2/10/2020


C-13 female
2/10/2020


C-25 female
2/10/2020


T-15 IM/UF male
2/11/2020

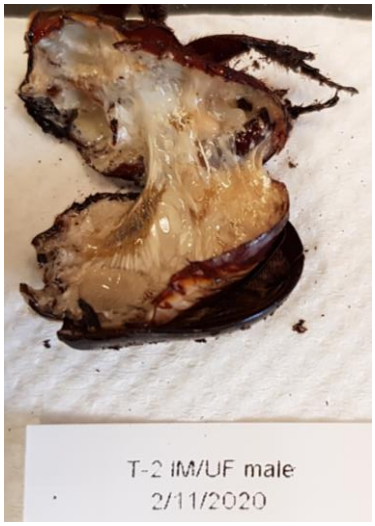

T-7 IM/UF male
2/11/2020


T-1 IM/UF female
2/11/2020


T-9 IM/UF male
2/11/2020


T-4 IM/UF female
2/11/2020


T-12 IM/UF female
2/11/2020


T-2 IM/UF male
2/11/2020


C-34 M/F male
2/11/2020


C-12 male
2/11/2020

C-32 M/F female
2/11/2020

C-31 M/F female
2/11/2020

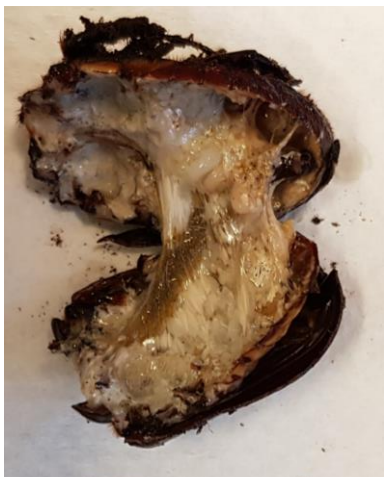
C-31 M/F male
2/11/2020

C-36 F/M male
2/11/2020

C-27 F/M male
2/11/2020

C-40 F/M female
2/11/2020

C-40 F/M male
2/11/2020



T-12 IF/UM male
2/18/2020

T-12 IF/UM female
2/18/2020


T-9 IF/UM female
2/18/2020


T-1 IF/UM female
2/18/2020

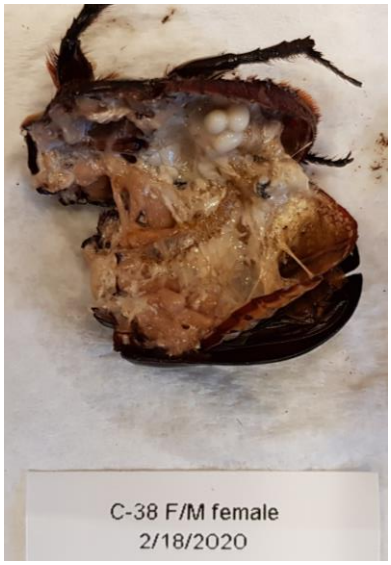

T-1 IF/UM male
2/18/2020


T-14 IF/UM male
2/18/2020


T-14 IF/UM female
2/18/2020


C-28 F/M female
2/18/2020


T-10 IF/UM male
2/18/2020


C-38 F/M female
2/18/2020


C-39 F/M female
2/18/2020