EXPERIMENTAL INFECTION OF WINTER WORKER BEES (*APIS MELLIFERA CARNICA*) WITH CHRONIC BEE PARALYSIS VIRUS (CBPV), STRAIN M92/2010

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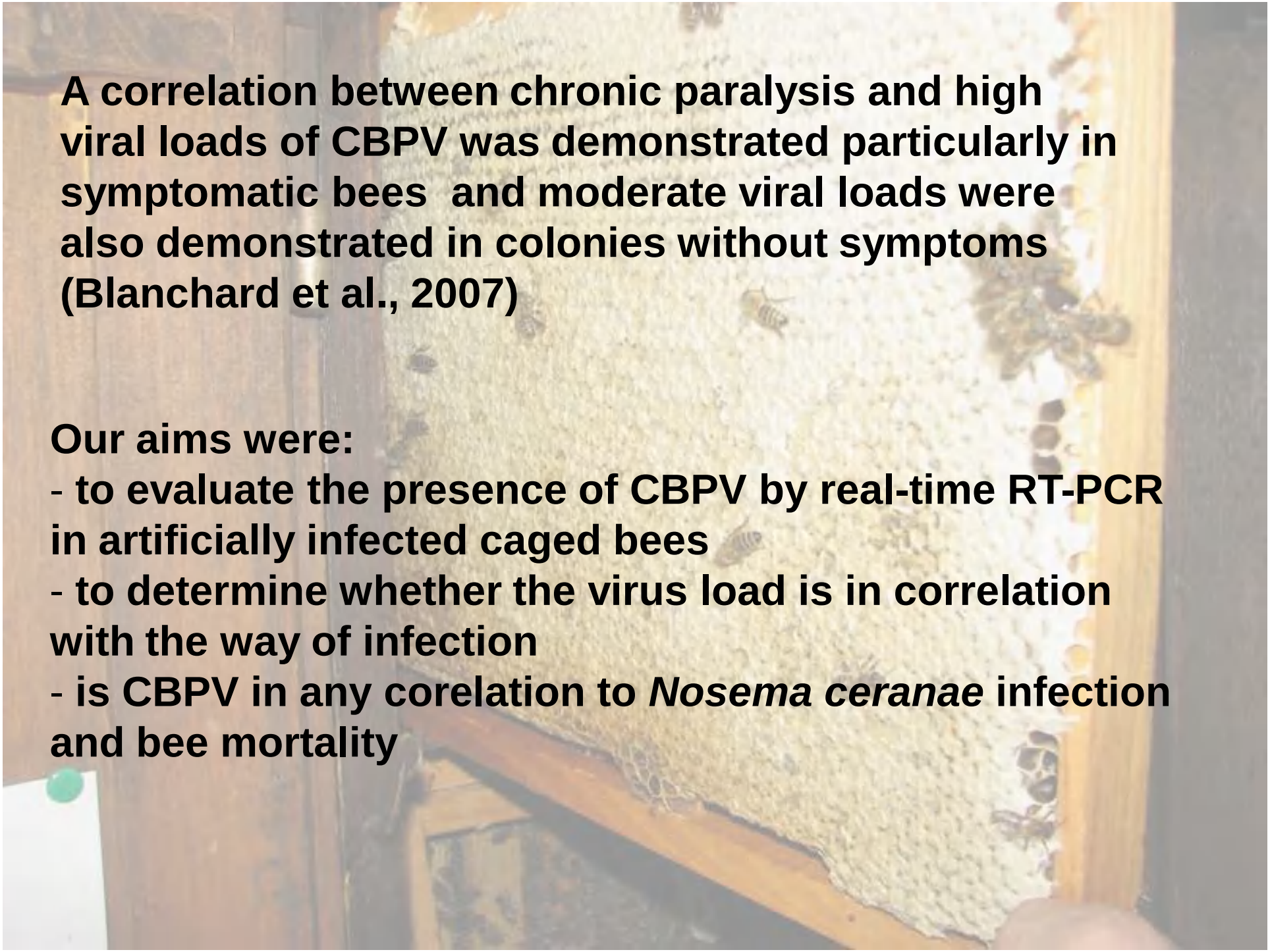
FIG 3B

Chronic bee paralysis virus (CBPV) is the causal agent of chronic paralysis known to induce significant losses in honeybee colonies (Ball and Bailey, 1997).

The pathology is characterized by clusters of trembling, flightless, crawling bees and by individual bees, sometimes hairless, standing at the hive entrance (Bailey et al., 1983).

The heads of bees naturally infected with chronic bee-paralysis virus contained about lOO times more virus per unit fresh weight than other body parts (Bailey, 1965)



A correlation between chronic paralysis and high viral loads of CBPV was demonstrated particularly in symptomatic bees and moderate viral loads were also demonstrated in colonies without symptoms (Blanchard et al., 2007)

Our aims were:

- to evaluate the presence of CBPV by real-time RT-PCR in artificially infected caged bees

- to determine whether the virus load is in correlation with the way of infection

- is CBPV in any corelation to *Nosema ceranae* infection and bee mortality

CBPV preparation and inoculation

The origin of CBPV strain 92/2010 derived from moribund bees.
Sample of 50 workers was tested for six honey bee viruses and was identified by RT-PCR method as positive on CBPV and BQCV and negative for ABPV, DWV, KBV and SBV.

Inoculations:

1. CBPV

-2µ CBPV suspension (contact)
-2µ CBPV suspension (p/o)
-Controls (RPMI)

Sampling: dead; live bees/3-5 days

2. CBPV + *Nosema*

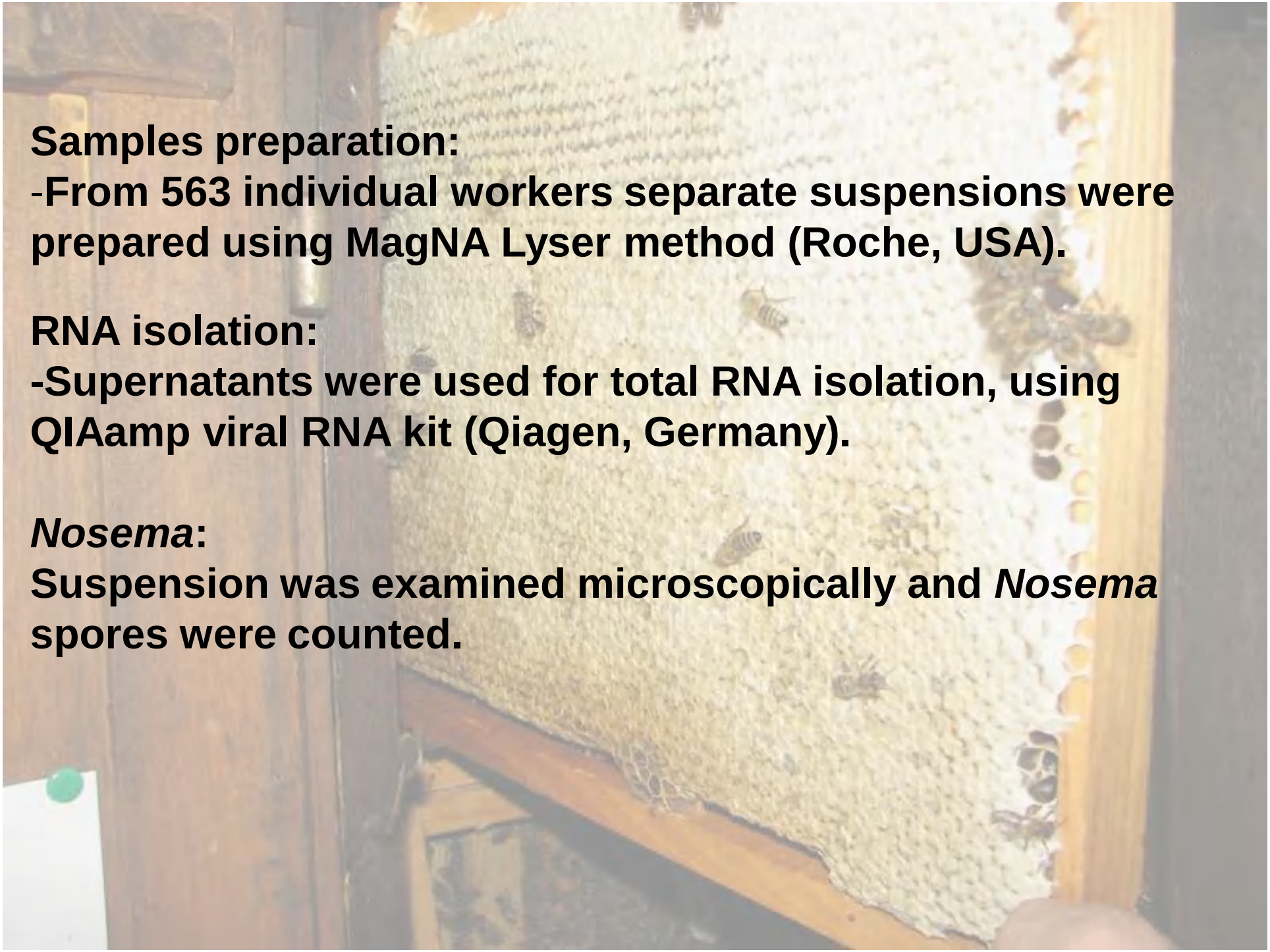
-2µl CBPV (contact) and 2µl Nos. (p/o) 60.000 spores
-2µl CBPV(p/o)+2µl Nos. (p/o)
-2µl RPMI+2 µl sugar sol. (p/o) – CONTROL

Sampling: live bees; dead bees; *Nosema* spores counting

3. Mortality test

- 2µl Nosema spores (p/o) + 2 µl sugar sol.
- 2µl CBPV (p/o) +2µl Nos.
-2µl CBPV (contact)
- 2µl CBPV (P/O) +2 µl sugar sol.
- 2µl RPMI (p/o) – CONTROL

Dead bees were sampled daily



Samples preparation:
-From 563 individual workers separate suspensions were prepared using MagNA Lyser method (Roche, USA).

RNA isolation:
-Supernatants were used for total RNA isolation, using QIAamp viral RNA kit (Qiagen, Germany).

***Nosema*:**
Suspension was examined microscopically and *Nosema* spores were counted.

Real-time CBPV:

- **The primers and probes for the real-time PCR (Blanchard et al., 2007).**
- **The TaqMan probe was labeled with the fluorescent reporter dye**

- **Real-time PCR was performed on MX3005P (Stratagene, USA).**
- **One–Step qRT-PCR was performed using Superscript™ III Platinum® kit with ROX (Invitrogen, GB) according to the manufacturer's instructions.**

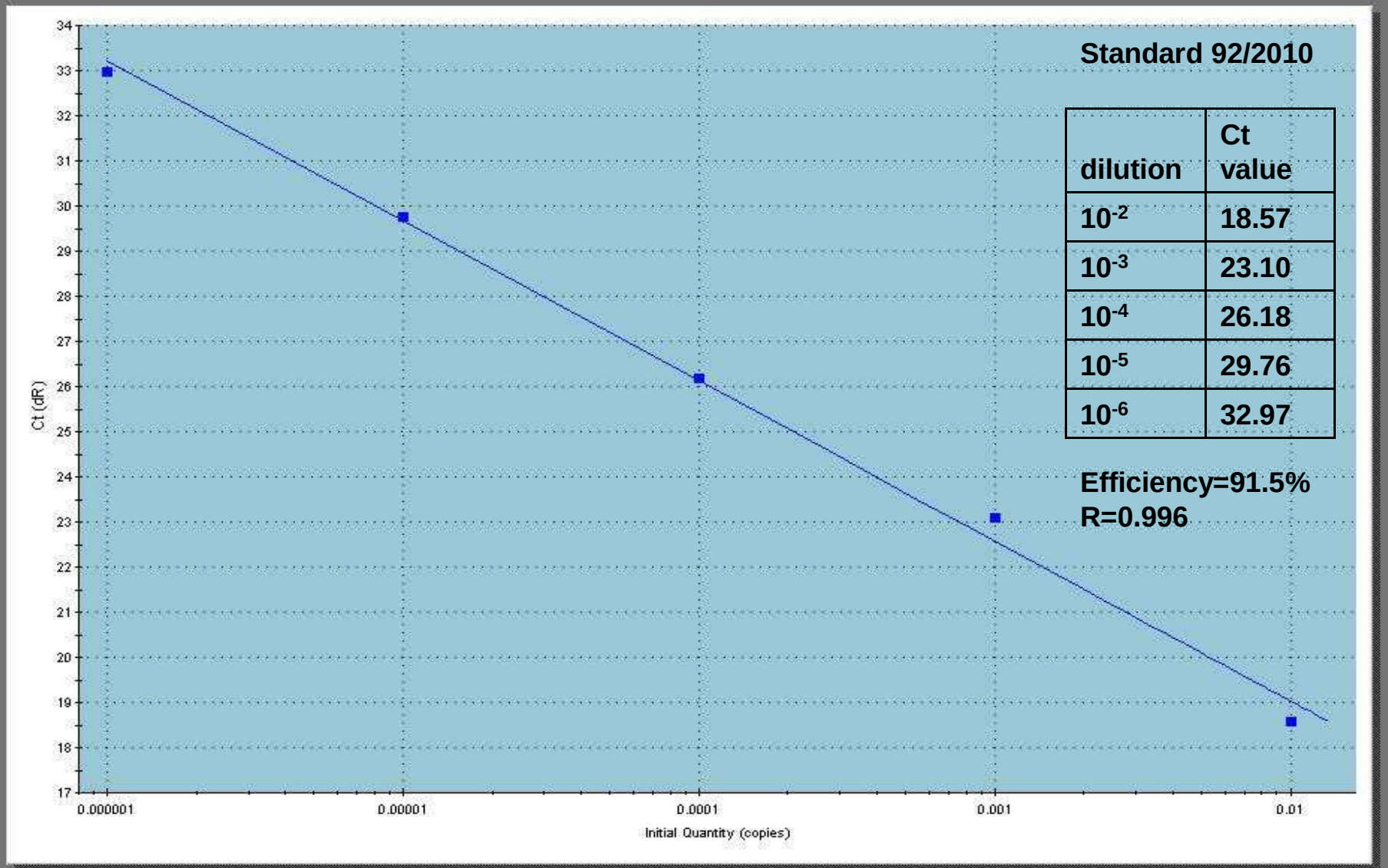
RNA assay optimization :

- **RNA volume (3 μL, 5 μL)**
- **number of cycles (40, 45)**
- **reaction mixture volume 15 μL and 25 μL**
- **the primer concentration and probe concentration remained unchanged (800 nM and 100 nM)**
- **annealing temperature was changed (50°C, 53°C, 55°C).**

After the optimization the RNA volume was 5 μL in 25 μL reaction mixture volume.

Thermal profile with the highest efficiency of the standard curve was used: 50°C for 15 min, 95°C for 2 min, followed by 45 cycles of 95°C for 15 s and 55°C for 1 min.

Standard Curve



Standard 92/2010

dilution	Ct value
10^{-2}	18.57
10^{-3}	23.10
10^{-4}	26.18
10^{-5}	29.76
10^{-6}	32.97

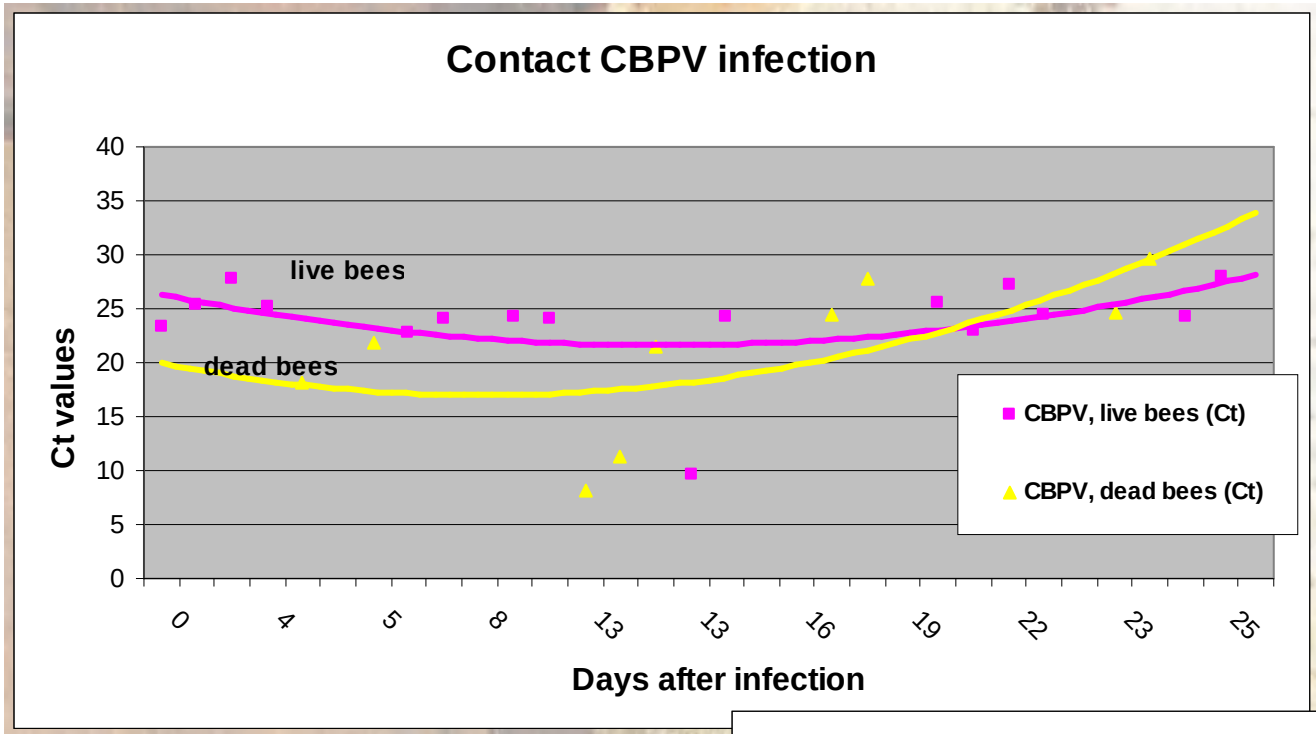
Efficiency=91.5%
R=0.996

Results

1. CBPV infections

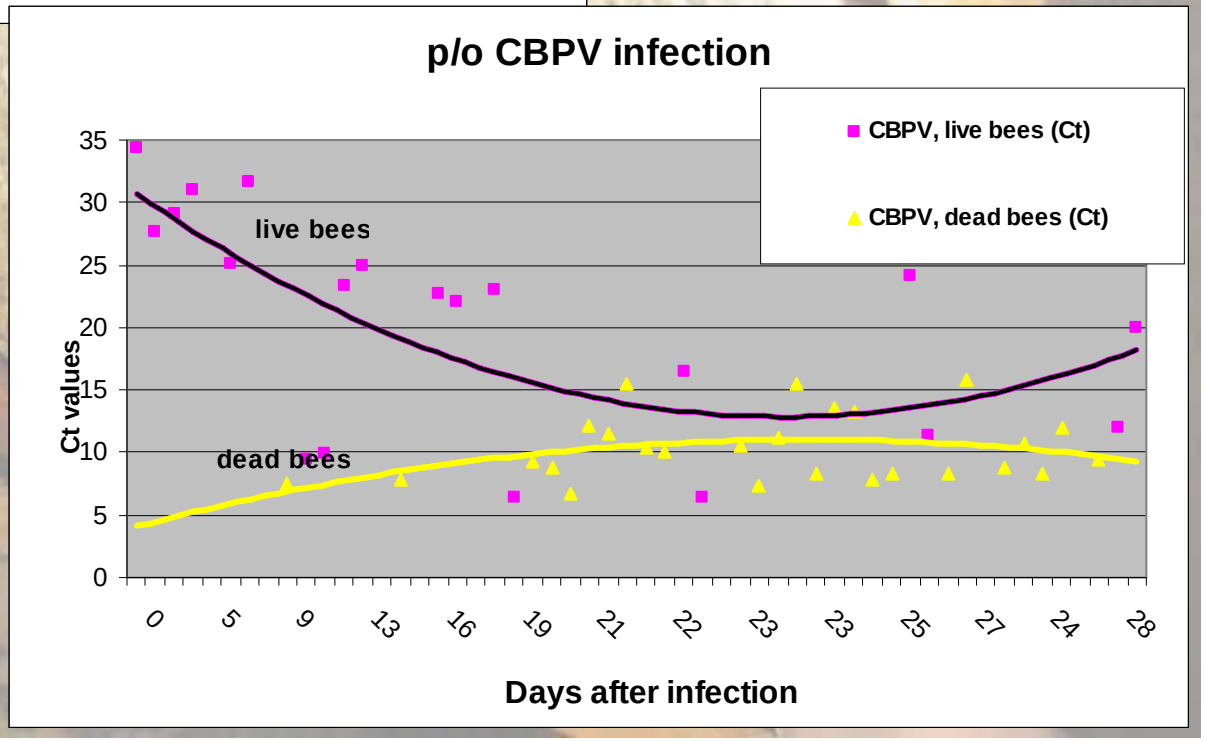
2µ CBPV suspension (contact)

2µ CBPV suspension (p/o)

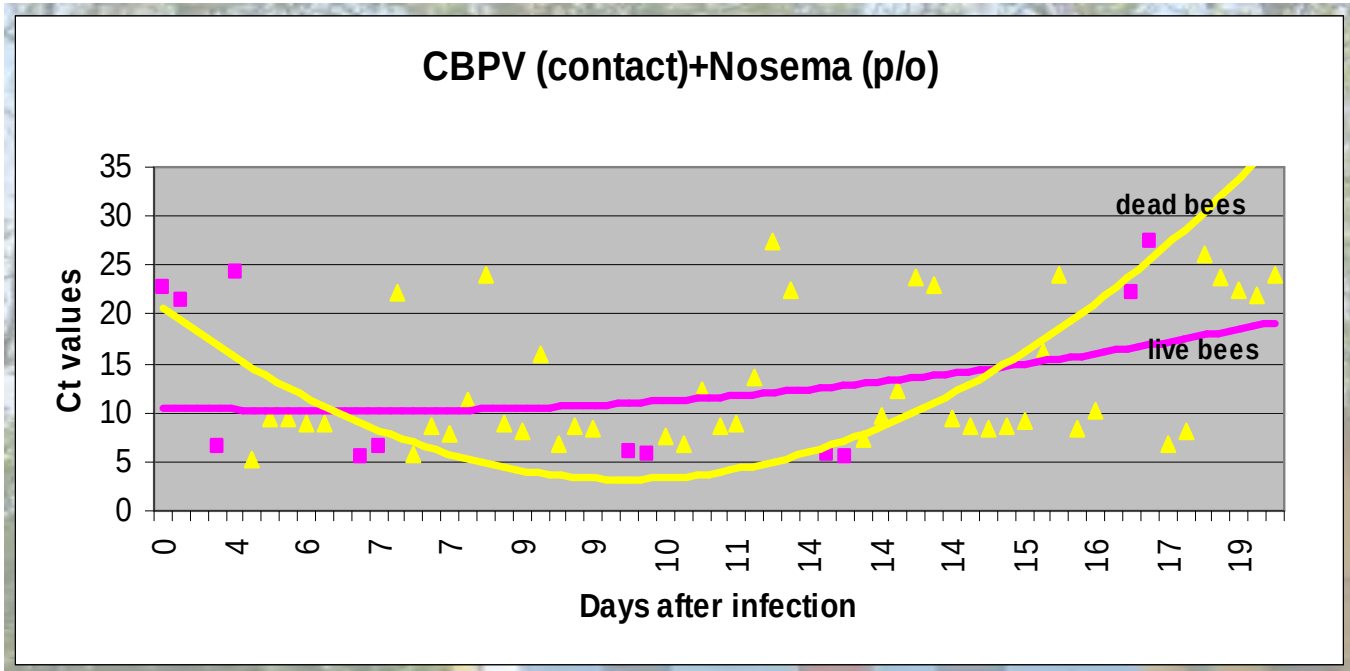


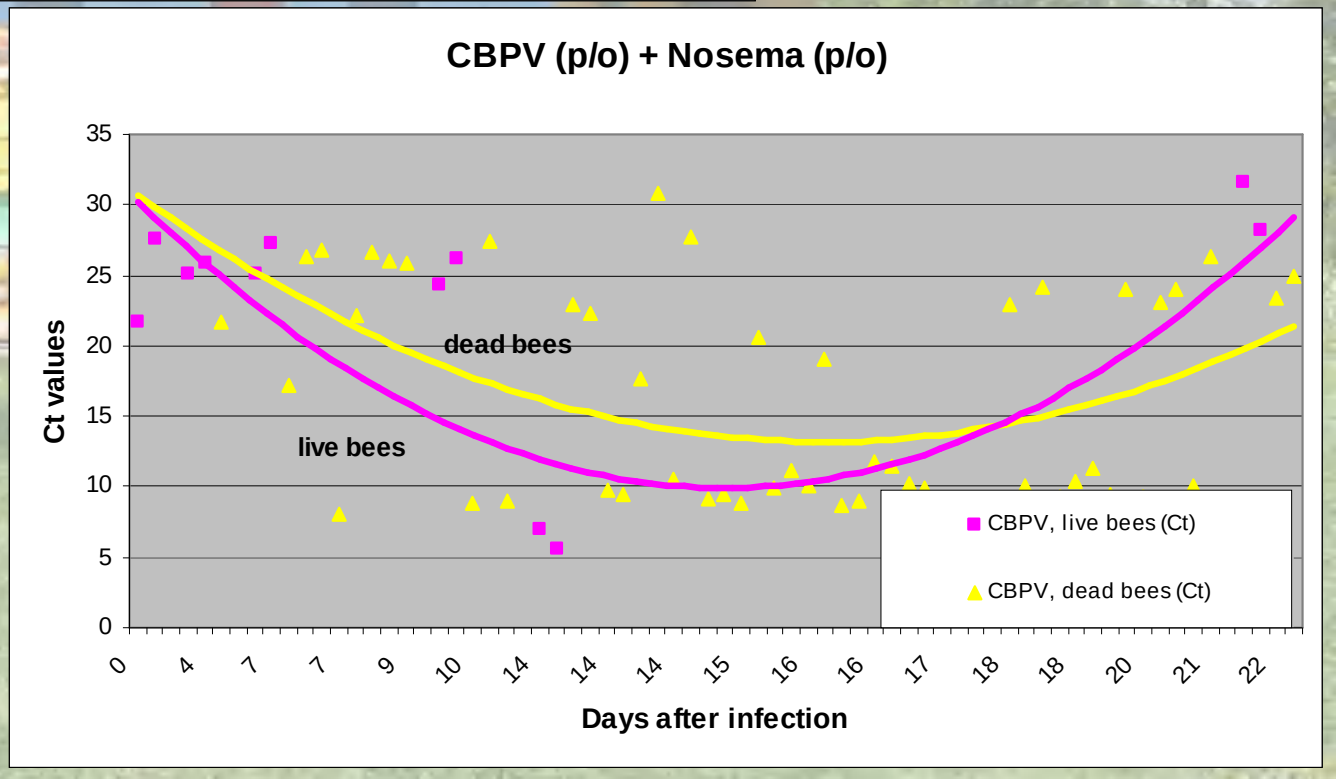
Ct value detected in inoculum was between

8 and 9

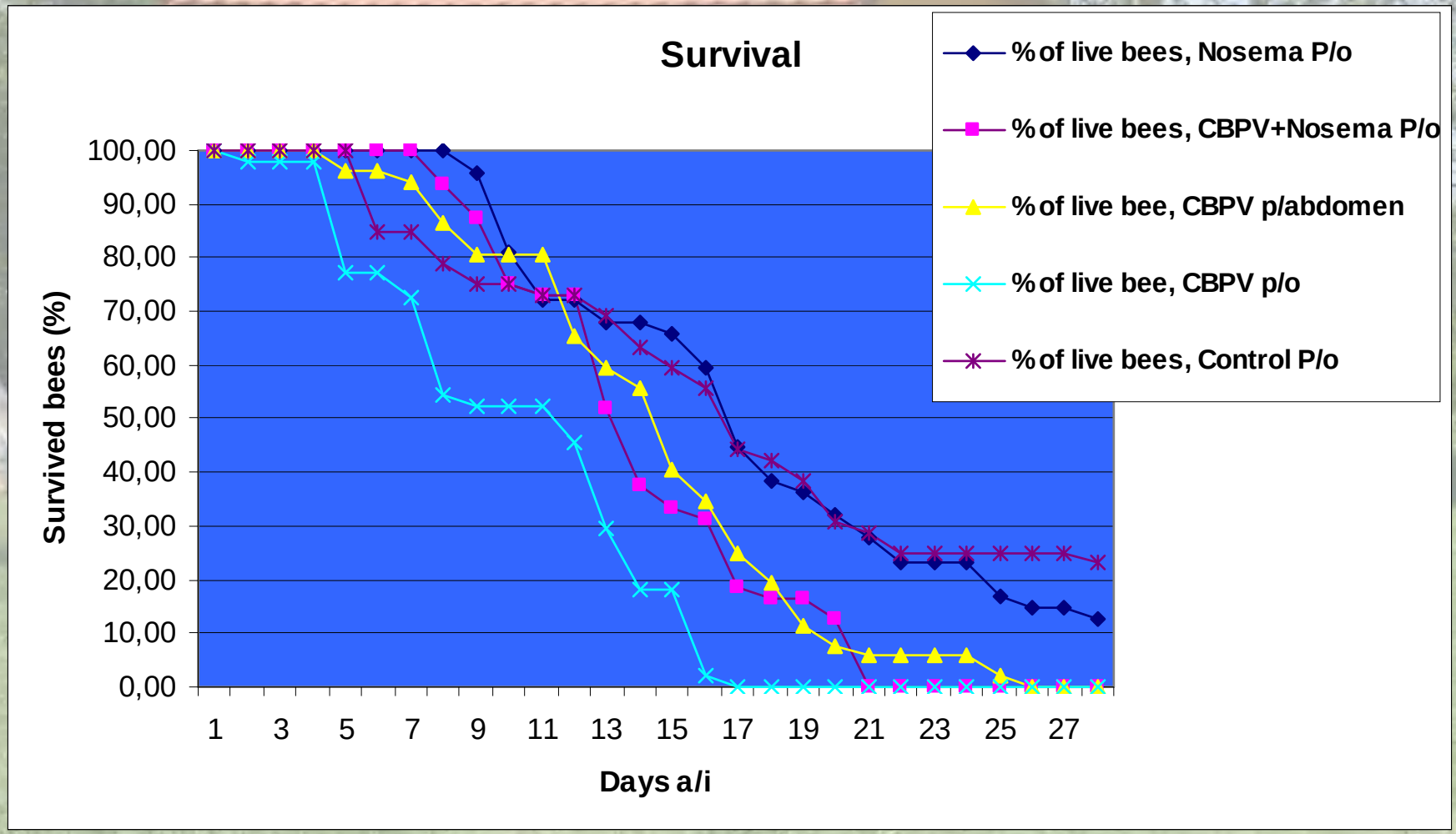


2. CBPV + *Nosema* inf.





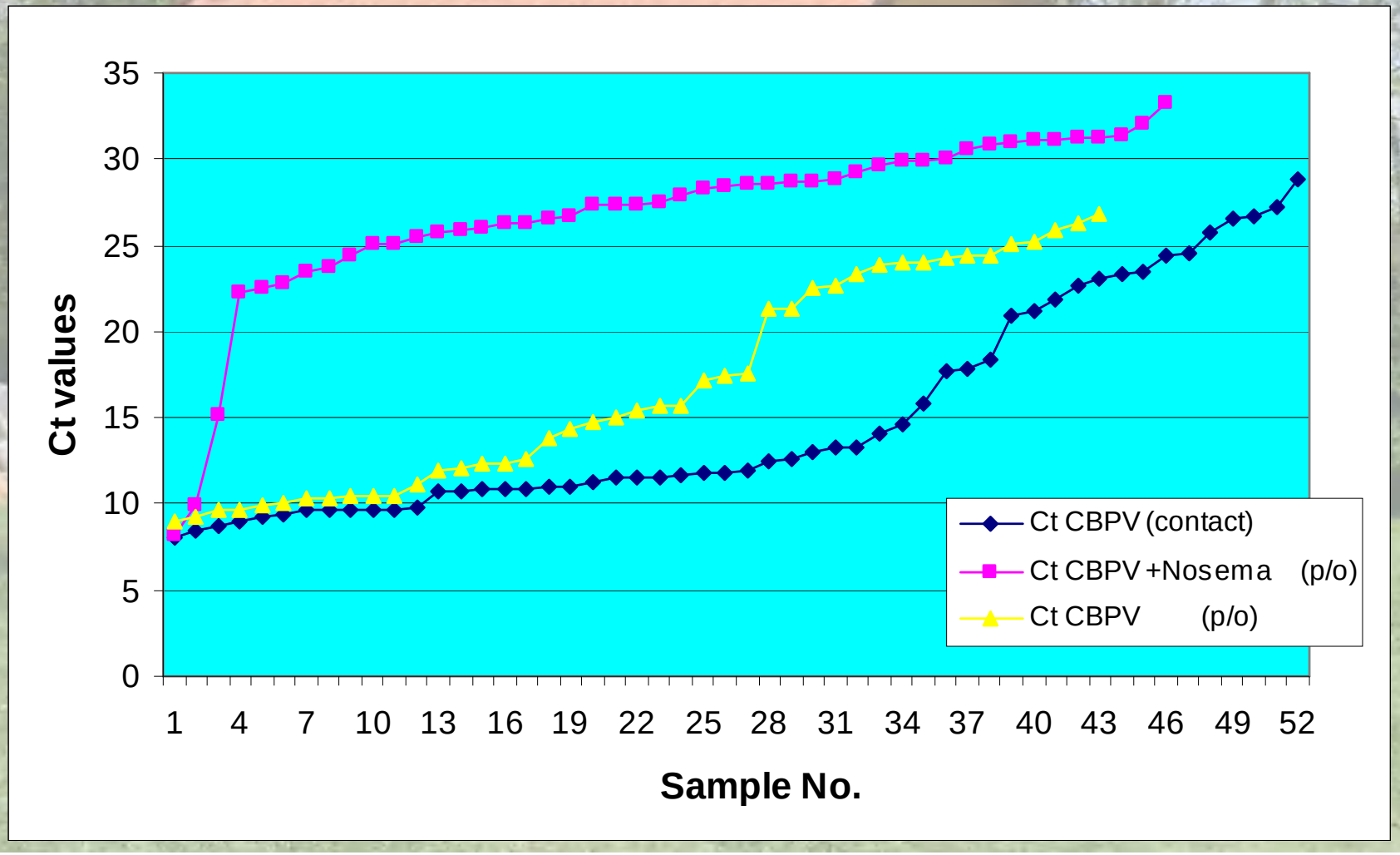
3. Mortality test **- 2µl *N. cerane* (p/o)** **- 2µl CBPV (p/o) + 2µl *N. ceranae* (p/o).**	**- 2µl CBPV (contact)** **- 2µl CBPV (p/o).** **- 2µl RPMI (p/o) – CONTROL** **Dead bees were sampled**



3. Mortality test (Ct values)

- 2µl CBPV (p/o) + 2µl N. ceranae (p/o).
-2µl CBPV (contact)
- 2µl CBPV (p/o).

Dead bees were sampled



Conclusions:

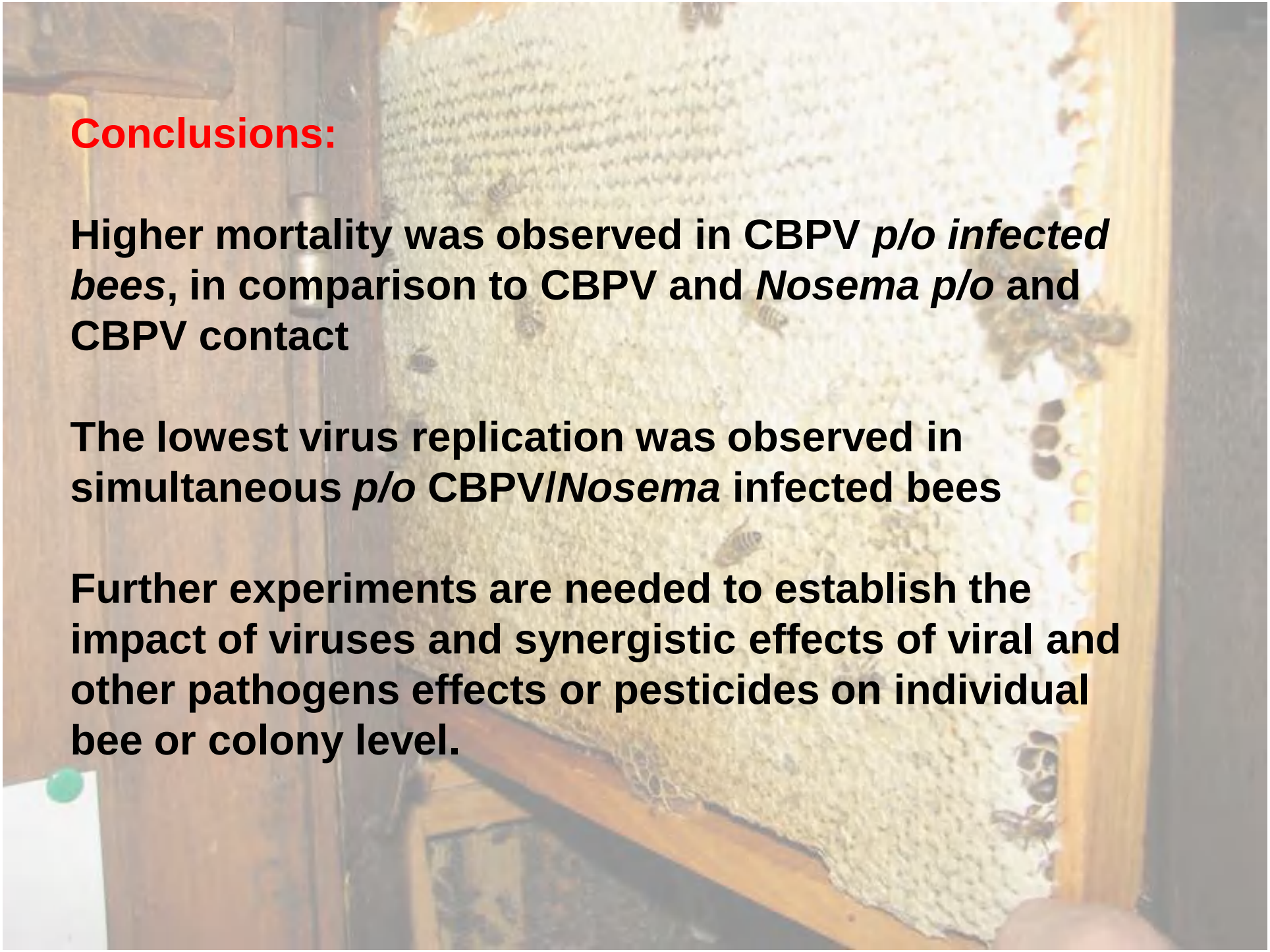
-winter worker bee infection with CBPV strain 92/2010 was demonstrated

-survived infected bees had lover virus load in comparison to dead bees

-*p/o* infection induced high virus load (8-28 p.i.) and bee mortality (acute infection).

- 1000 X higher virus load was detected (Ct in dead compared to Ct of live bees) in 28 day experiment (CBPV *p/o*, without *Nosema*)

- simultaneous contact CBPV infection&*Nosema* (*p/o*) induced higher virus load in comparison to *p/o* virus infection



Conclusions:

Higher mortality was observed in CBPV *p/o infected bees*, in comparison to CBPV and *Nosema p/o* and CBPV contact

The lowest virus replication was observed in simultaneous *p/o* CBPV/*Nosema* infected bees

Further experiments are needed to establish the impact of viruses and synergistic effects of viral and other pathogens effects or pesticides on individual bee or colony level.


Thank you for your attention !