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Scientific note

A scientific note on the toxic pollen of *Stryphnodendron polyphyllum* (Fabaceae, Mimosoideae) which causes sacbrood-like symptoms

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Since 1983, many samples of diseased brood, mainly from southeastern states, have been diagnosed as sacbrood-like disease in Brazil. This disease has caused serious losses to beekeepers who in some cases, have lost all their colonies in less than one month (Message, 1997).

The main symptoms are the following: the larvae fail to pupate and remain stretched on their backs, with their heads towards the cell capping. Fluid then accumulates between the body of the diseased larva and its unshed skin, and the body color of the larva changes from pearly white to a pale yellow. After it has died, it becomes dark brown over a few days. The head and thoracic regions darken first, and finally the larva dries down to a flattened gondola-shaped scale, as described by Bailey and Ball (1991) for the sacbrood disease caused by the 30 nm viral particle SBV (Bailey et al., 1964).

All possible means to purify the causative agent were used in Brazil from 1983 until 1995. From May 1995 to July 1996, we used Bailey and Ball's method (1991) for the extraction and purification of bee viruses at Rothamsted (UK). The pellets obtained were submitted to a double immunodiffusion test for different bee viruses and also observed in negatively stained preparations with electron microscopy (Bailey and Ball, 1991). No virus particle or other microorganism was detected in the diseased brood from Brazil with sacbrood-like symptoms. Also, injection of ecdysial fluid from this diseased brood into healthy white-eyed pupae did not result in any virus multiplication (Message et al., 1995). Thereafter, the disease was named Brazilian sacbrood-like disease (BSBD) by Message (1997). Preliminary epidemiological observations led to the hypothesis that pollen sources might be the causative agent of BSBD symptoms.

To test this hypothesis, 3 experiments were conducted on worker brood reared in the laboratory using a technique developed by Vandenberg and Shimanuki (1987), with some adaptations introduced by Silva (1995). For the first two daily feedings, the following diet formulation was given to worker larvae: royal jelly (10 g), water (7.4 cm^3), D-Fructose (1.4 g), D-Glucose (1.4 g) and yeast extract (0.2 g) and for the three subsequent feeding days, we used the same diet and added 0.5% (w/w) experimental pollen.

In a preliminary experiment under laboratory conditions, stored pollen from the combs (bee bread) of colonies severely affected by BSBD was used as treatment while bee bread from another geographical region not affected by BSBD was used as control. The larvae from the treated group developed symptoms typical of BSBD (Fig. 1a), similar to diseased brood in the field (Fig. 1b), while none of the brood in the control group developed such symptoms. These preliminary results indicated that pollen could be the causative agent, but it could not be ascertained which pollen was toxic because the bee bread used was a mixture of different pollens.

In a second experiment, pollen pellets were collected with pollen traps during an outbreak of BSBD. Four main pollen types sorted by color and morphology were tested against a control diet without pollen and a control diet with pollen from a non epidemic region. Only one of the pollen species tested caused brood mortality with typical symptoms. The pollen pellets containing this species had a greenish-brown color. The large sized pollen showing a polyad morphology with 16 associated grains was compared with reference pollen collection made from plants that were flowering in

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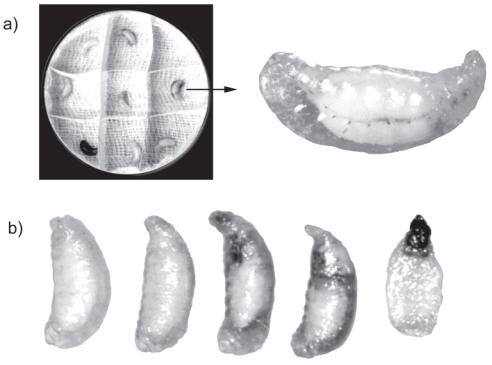

Figure 1. (a) Typical symptoms of Brazilian sacbrood-like disease (BSBD) in larvae fed with a diet containing *Stryphnodendron polyphyllum* ("barbatimão") pollen in laboratory conditions, (b) typical symptoms of Brazilian sacbrood-like disease in Africanized honeybees colonies during a "barbatimão" blooming.

the region during the disease outbreak and the pollen was identified as that of *Stryphnodendron polyphyllum* Martius ("barbatimão"). Guinet and Caccavari (1992) described *S. polyphyllum* v. *villosum* Benth polyad as containing 16 grains (90%) or 8 grains (10%) and the longest/shorter polyad axis in broad view as 34.2/24.2 µm and 35.0/24.0 µm.

In a third experiment we repeated the previous protocol using more replicates and pellets of pure *S. polyphyllum* pollen. In the treatment group, a high proportion of brood (85%) failed to pupate and the prepupae had typical BSBD symptoms, while 78% of the larvae were able to pupate in the control group fed with a mixed pollen from pellets without barbatimão pollen ($P = 0.006$, n = 6, Mann-Whitney U test). Silva (1995) obtained 84% successful pupation when she used a similar diet without toxic pollen. It is noteworthy that there has been no reported pesticide applications from the different regions where the toxic pollen was collected.

Various species of *Stryphnodendron* bloom during different periods from September to March in Brazil, but until now we observed larvae mortality by BSBD in areas containing only two of them, *S. polyphyllum* and *S. adstringens* (Mart.) Coville. Further investigations on the effects of other "barbatimão" species are required. BSBD seems to incur more losses for beekeepers when climatic factors reduce other sources of pollen, inducing bees to forage mainly on the toxic pollen. Based on our field observations, brood is less affected when foragers have access to other pollen sources so that toxic pollen is diluted with others. The toxic substances in *Stryphnodendron* pollen, as well as variations in brood mortality among different colonies and within the same colonies at different times, are now being studied.

Beekeepers have been advised to move their bee colonies to other regions free of "barbatimão" trees when these are in bloom and also to not use antibiotic treatments when BSBD symptoms occur, since no microorganisms is involved.

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Note scientifique sur le pollen toxique de *Stryphnodendron poplyphyllum* (Fabaceae, Mimosoideae) qui provoque des symptômes ressemblant au couvain plâtré.

Eine wissenschaftliche Notiz über toxischen Pollen von *Stryphnodendron polyphyllum* (Fabaceae, Mimosoideae), der Sackbrut ähnliche Symptome verursacht.

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