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The potential of silicate rock dust to control pollen beetles (*Meligethes* spp.)

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Abstract: In organic agriculture, treatments with rock dusts for soil amendment, disease prevention, or insect control have a long tradition. Clinoptilolite (a naturally occurring zeolite) was tested against pollen beetle *Meligethes* spp. in organic and IPM oilseed rape fields in order to find a control strategy compatible with the guidelines for organic and IPM agriculture in Switzerland. Dust and spray applications were evaluated in several large-scale field trials from 2008 to 2011. Dust applications using 300-750 kg/ha pulverized Clinoptilolite (particle size < 0.1 mm; product Klinofeed, available from Unipoint, Switzerland) were applied using a drop-box fertilizer spreader or a Vicon pendulum spreader. For spray applications the same Clinoptilolite (product Klinospray) with a particle size of 0.017 mm was applied at rates of 30-50 kg/ha with a wetting agent (Heliosol, 2 l/ha; 600 l waster/ha) using a standard field crop sprayer. Two or three applications were conducted during the inflorescene stage (BBCH 51-57) depending on the rate of development of the oilseed rape plants.

Under the dry and sunny weather conditions in 2009 and 2010, the treatments significantly reduced the number of pollen beetles by 50 to 80% until seven days after treatment (BBCH 51-54). Under the rainy weather conditions in 2008, no reduction of pollen beetles was observed. However, pollen beetles in treated plots showed a lower activity compared to beetles from the untreated control plots. In all experimental years flowering was visibly more intense in the treated than control plots. Pod setting on the main raceme was significantly increased in the treated plots. The yield was significantly increased by 23% in the experiments conducted under IPM conditions in 2010. However, no yield increase was observed in 2008 and 2009 under organic agricultural conditions.

Key words: *Meligethes* spp., organic agriculture; stone meal; rock dust; clinoptilolite; zeolite

Introduction

In organic agriculture, methods to control pollen beetle (*Meligethes* spp.) are limited. Although effective organic insecticides are available (e.g. Spinosad), their use is often restricted by guidelines of producers associations. In Swiss organic production (Bio Suisse), as well as in Swiss IPM-production (IP-SUISSE) the use of insecticides in oilseed rape and cereals is restricted. Therefore, alternative non-insecticidal methods to control pollen beetles are needed.

Treatments with rock dusts for soil amendment, disease prevention or insect control have a long tradition in organic agriculture. However, little research was conducted to investigate efficacy and mode of action of different inert dusts (Ebling & Wagner, 1959, Ulrichs *et al*., 2006, Wagner & Ebling, 1959). Only the use of amorphous silica (diatomaceous earth) for pest regulation in stored products is well studied (Golob, 1997). It was shown that diatomaceous earth does not kill by poisoning or suffocation, but by desiccation: the protective wax layer covering the insect's body is lost either by abrasion or absorption, leading to an increased water loss and consequently death of the insect (Ebling & Wagner, 1959, Ulrichs *et al*., 2006). By comparing different abrasive and sorptive dusts, Ebling &

Wagner (1959) and Ulrichs *et al*. (2006) showed that sorptive dusts were more effective than abrasive dusts.

Another well-studied inert dust used for pest control is kaolin-clay (Glenn *et al*., 1999). The particle film technology using kaolin was developed in the 1990's and is now used against different pest insects in perennial crops (Bürgel *et al*., 2005, Daniel *et al*., 2005, Saour & Makee, 2004). It is assumed that processed-kaolin particle film technology does not kill insects, but has a repellent and/or barrier effect: Plants may become unrecognisable (by affecting visual and/or tactile host-location cues), and feeding and oviposition may be impaired by the attachment of particles to the bodies of the insects as they crawl upon the film (Glenn *et al*., 1999, Wyss & Daniel, 2004). References on other inert dusts for pest control are few (Humphrys & Jossi, 2009, Ulrichs *et al*., 2006, Wagner & Ebling, 1959).

Clinoptilolite is a naturally occurring Zeolite: a microporous, aluminosilicate mineral commonly used as commercial adsorbent or deodorizer. In agriculture, Clinoptilolite is used as feed additive in animal production or as soil conditioner. No studies are available on the effects of Clinoptilolite for pest control. In this series of experiments the efficacy of Clinoptilolite to control pollen beetle (*Meligethes* spp.) was investigated.

Material and methods

Experiments were conducted in Switzerland on commercial organic and IPM farms. Large-scale experiments were set up to monitor the migration of pollen beetle an compare between untreated plots and plots treated with the test materials using standard application equipment.

Experiments in 2008

In 2008, the first pilot experiments were conducted in seven organic winter oilseed rape fields (total area: 4.2 ha) in North-western Switzerland. One half of each field was treated, the other half remained untreated. In the two largest fields, two replicates were set out (two treated and two untreated plots per field). Treated plots had an average size of 0.3 ha (0.15-0.40 ha).

Inert dust can either be sprayed or dusted onto the crop. Dust applications were conducted in six fields using a Vicon pendulum spreader, or a drop-box-fertiliser-spreader. The product Klinofeed (Clinoptilolite; Unipoint, Switzerland; particle size: 90% < 100 µm) was applied at rates of 300-750 kg/ha per application. Spray applications of Klinospray (Clinoptilolite; Unipoint, Switzerland; particle size: 17 µm; 50 kg/ha) in combination with a pinolene-based wetting agent (Heliosol; Omya AG Agro, Switzerland; 2 l/ha) were conducted in one field (two replicates) with a standard field sprayer using 600 l water/ha. The first applications were applied when buds were visible (stage 51 BBCH). Two or three treatments were applied during blossom development in order to compensate for washing off by rainfall. The last application was applied at growth stage 56-59.

Experiments in 2009

In 2009, one experiment was conducted in an organic winter oilseed rape field (area: 3.8 ha) in the same region in North-western Switzerland as in 2008. Four replicates were set up in a block design. Treated plots were 0.48 ha. The product Klinofeed (300 kg/ha) was applied at growth stage 51 BBCH (15.04.2009) and at growth stage 58 BBCH (25.04.2009) using a drop-box-fertiliser-spreader.

Experiments in 2010

The experiments in 2010 were conducted in six IPM winter oilseed rape fields (total area: 9.0 ha) in North-western and South-western Switzerland. One or two replicates per field were set up (total: 7 replicates). Treated plots had an average size of 0.36 ha (0.17-0.6 ha). Because the application technique for dust applications was not available on IPM-farms, spray applications of Klinospray (30 kg/ha + wetting agent Heliosol 2 l/ha, with 600 l water/ha) were applied at growth stage 51-52 BBCH, 53 and 55-56 (only fields in North-western Switzerland with high infestation pressure).

Sampling

All sampling was done at five sampling points in the centre of each plot. At each sample point, five plants were selected at random and the number of adult pollen beetles per plant was assessed by beating the plant over a plastic tray. Assessments were conducted around noon (10:00-14:30 o'clock) from growth stage 50-60 BBCH. In Mid-June, plant density was determined by counting the number of plants per m^2 at each sampling point. Plant samples were taken (3 plants per sampling point) by cutting the plants at ground level. Assessments included fresh weight per plant, stem-diameter at ground level, plant height, number of side shoots, pods per main shoot, pods per plant, pods damaged by pod midge, number of podless stalks (damage by pollen beetles) per main shoot, length of galleries inside the stem (damage of stem mining insects). Fresh weight and pods per m^2 were calculated.

Statistical analysis

Data were analysed using the software JMP5.0.1. Data from 2008 and 2010 were tested for normal distribution and homogeneity of variances and analysed by two-way ANOVA [factors: treatment, field]. Data from 2009 were [$\sqrt{(x)}$]-transformed to obtain normal distribution and homogeneity of variances and then analysed by one-way or two-way ANOVA [factors: treatment, block]. Data given in figures and text are means with standard deviations.

Results

Experiments in 2008

Climatic conditions, plant development, and the start of pollen beetle immigration are given in Table 1.

Under the very wet and rainy conditions in 2008, only Klinofeed at the highest application rate (750 kg/ha) significantly reduced the number of beetles per plant one day after treatment (3.1 ± 2.0 beetles/plant) compared to the untreated control (6.5 ± 3.0 beetles/plant; efficacy: 48%). A lower application rate (350 kg/ha) resulted in 6.0 ± 2.3 beetles/plant (efficacy: 8%; two-way ANOVA [application rate, field], $F_{2,13} = 7.0$, $p = 0.009$, Tukey HSD-test). Three days after treatment, the effect of the highest application rate was no longer observed (two-way ANOVA [application rate, field], $F_{2,13} = 2.7$, $p = 0.1$).

Spray applications of Klinospray at a rate of 50 kg/ha did not reduce the number of beetles per plant one day after application (control: 9.6 ± 0.4 beetles/plant; Klinospray: 12.9 ± 1.1 beetles/plant).

Observations during sampling indicate that the beetles in the Klinofeed treated plots were covered in white powder and were visibly less active than beetles in the control plots. In the Klinospray treated plots, a similar reduction in activity was observed; however, it was less pronounced. In the plots treated with Klinofeed, flowering started about 5 days earlier than in the control plots.

Five of the seven fields were in very bad condition due to the cold, rainy weather leading to limited nitrogen mineralization from the organic fertilizer applied. These plots were therefore ploughed-up during flowering. Only the two largest fields remained until harvest (one field treated with Klinospray, one field treated with Klinofeed; both with two replicates per field). The Number of pods per main shoot was increased by 83% after three applications with 750 kg Klinofeed and by 58% after three treatments with 50 kg Klinospray. The number of pods per plant was increased by 52% (Klinofeed) and 14% (Klinospray), respectively. The number of pods per m^2 was increased by 82% (Klinofeed) and 37% (Klinospray), respectively.

Table 1. Plant development, first observations of *Meligethes* spp. on winter oilseed rape plants and climatic conditions during April in the years 2008-2010

Year	**2008**	**2009**	**2010**
Plant development			
BBCH 51	05.04.-15.04.	03.04.-08.04.	07.04.-14.04.
BBCH 61	01.05.-05.05.	21.04.-10.05.	25.04.-30.04.
First observations of Meligethes on oilseed rape plants and average number of Meligethes per plant at stage 51, 52-53, and 57-58 BBCH			
First observations	15.02.	03.04.	19.03.[a] (17.04.[b])
Meligethes / plant:			
stage 51 BBCH	2.6	0.8	3.4[a] (0.2[b])
stage 52-53 BBCH	5.3	4.6	15.5[a] (2.9[b])
stage 57-58 BBCH	8.3	2.6	11.2[a] (3.4[b])
Climatic conditions during April			
Mean Temperature °C	8.6	12.1	10.5
Min. Temperature °C	-3.2	2	-0.7
Max. Temperature °C	21	23.4	26.6
Precipitation (mm)	202.2	19.6	32.4

[a]fields in north-western Switzerland 2010; [b]field in south-western Switzerland 2010 ;

Experiments in 2009

In 2009, Klinofeed (dust application, 300 kg/ha, BBCH 51-52) was applied under dry and sunny weather conditions. One day after treatment, the number of beetles per plant was significantly reduced by 73% (Figure 1). Nine (rainless) days after treatment at growth stage 58 BBCH, efficacy was 78% (Figure 1). Plant samples were taken after flowering to determine pod set. Damage by pollen beetles (podless stalks) was significantly reduced by 17% (Figure 1).

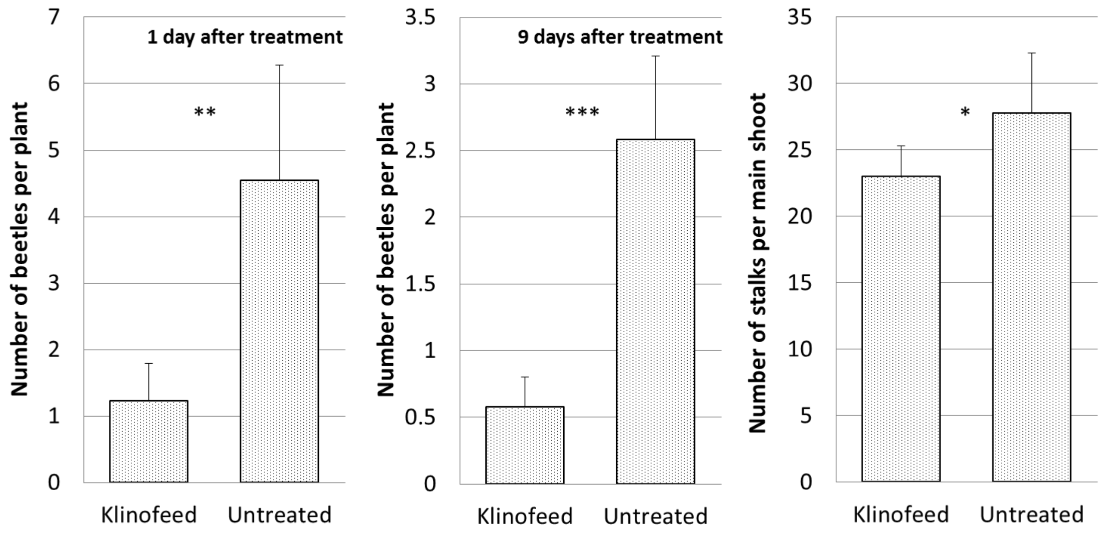

Figure 1. Effect of Klinofeed dust applications on number of pollen beetle one (BBCH 52) and nine days (BBCH 58) after application, and pollen beetle damage (podless stalks) per main shoot in 2009. Statistics: data transformed [$\sqrt{(x)}$]; One day: one-way ANOVA, $F_{1,6}$ = 17.8 p = 0.006; Nine days: one-way ANOVA, $F_{1,6}$ = 45.6, p = 0.0005; stalks: two-way ANOVA [treatment, block], $F_{1,3}$ = 10.8, p = 0.046.

Numbers of pods per main shoot and per plant were increased by 46% and 34%, respectively (Figure 2). The number of pods per m^2 was significantly increased by 100% (Figure 2). However, no increase in yield was observed at harvest; treated plots yielded 1130 ± 130 kg / ha, untreated plots yielded 1150 ± 260 kg / ha. These results indicate that nitrogen availability instead of the pollen beetle damage was the yield-limiting factor under the organic agricultural conditions in 2009.

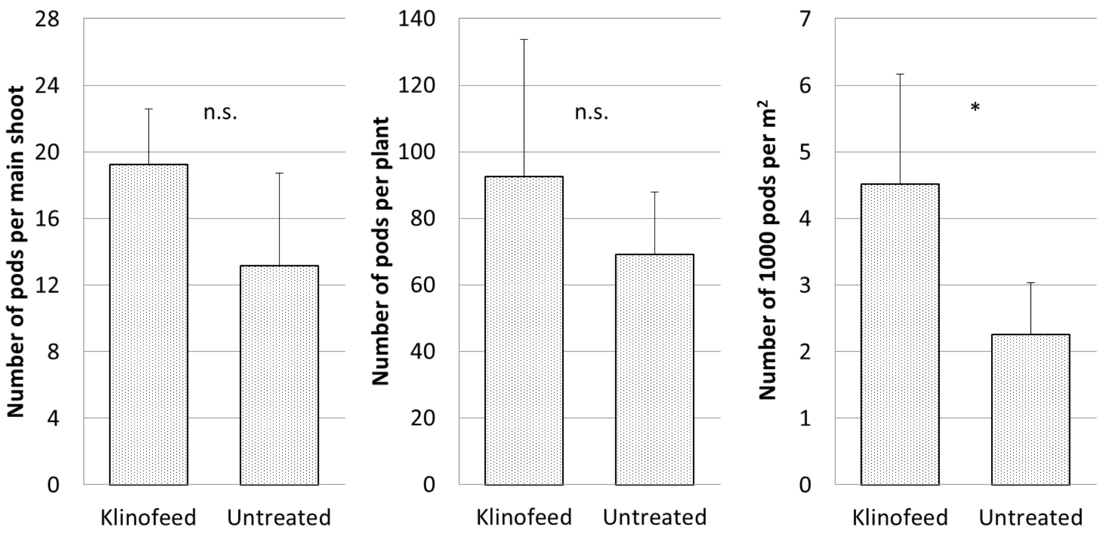

Figure 2. Effect of Klinofeed dust applications on the number of pods per main shoot, per plant and per m^2 in 2009. Statistics: data transformed [$\sqrt{(x)}$], Two-way ANOVA; main shoot: $F_{1,3}$ = 8.07, p = 0.067; plant: $F_{1,3}$ = 4.3, p = 0.13; m^2: $F_{1,3}$ = 14.1, p = 0.03.

Experiments in 2010

Experiments in 2010 were conducted on IPM fields with a considerably higher nitrogen supply. In addition, infestation pressure with pollen beetle in 2010 was substantially higher than in 2009 (Table 1). Similar to the conditions in 2009, the weather remained sunny and almost rainless during the whole experimental period until start of flowering (growth stage 60 BBCH). Spray applications of 30 kg Klinospray/ha at growth stage 51 BBCH significantly reduced the number of beetles per plant and resulted in an efficacy of 52%, 45% and 50% one day, three days and seven days after the first application, respectively (Figure 3). Three days after the second treatment, efficacy was 22% (Figure 3). Five days after the last application (BBCH 59), 23% more pollen beetles were observed in the treated plots as compared to the untreated control (Figure 3). This observation might be due to the near-complete damage of the untreated plots: beetles might have left the control plots in search of flowering plants.

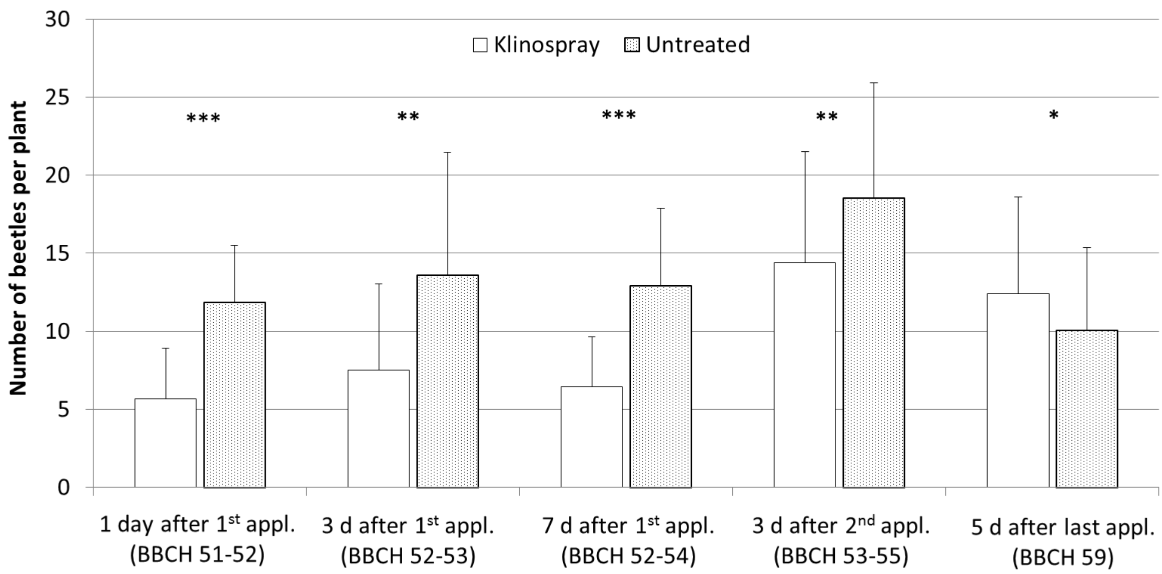

Figure 3. Effect of Klinospray applications on the number of pollen beetles per plant in 2010. Statistics: two-way ANOVA [treatment, field]; BBCH 51-52: $F_{1,5} = 53.2$, $p = 0.0008$; BBCH 52-53: $F_{1,6} = 23.5$, $p = 0.003$; BBCH 52-54: $F_{1,6} = 52.9$, $p = 0.0008$; BBCH 53-55: $F_{1,6} = 19.1$, $p = 0.005$; BBCH 59: $F_{1,6} = 6.8$, $p = 0.04$.

Infestation levels differed substantially between experimental fields leading to large standard deviations (Figure 3). In south-western Switzerland, the number of beetles per plant remained below 5 for the whole experimental period, whereas in north-western Switzerland an average of 25 beetles per plant was observed at growth stage 54 BBCH. Nevertheless, efficacy was similar in all experimental fields.

The number of pods per main shoot was significantly increased by 53% in the Klinospray treated plots. The number of pods per plant and per m^2, however, were only increased by 9% and 11%, respectively (Figure 4).

Yield at harvest was significantly increased by 23% (two-way ANOVA, one field excluded because of heavy boar damage; $F_{1,4} = 8.4$, $p = 0.04$). Treated plots yielded 1380 ± 1260 kg/ha, untreated plots yielded 1120 ± 1170 kg/ha. Yield varied greatly between the different regions: with the low beetle pressure in South-western Switzerland, a yield of 3170 kg/ha was obtained from the untreated controls. Treatments with Klinospray increased the yield by 11% (3520 kg/ha). With the high beetle pressure in North-western Switzerland, yield was considerably lower (610 kg/ha in the untreated plots). Treatments resulted in a 37% yield increase (840 kg/ha).

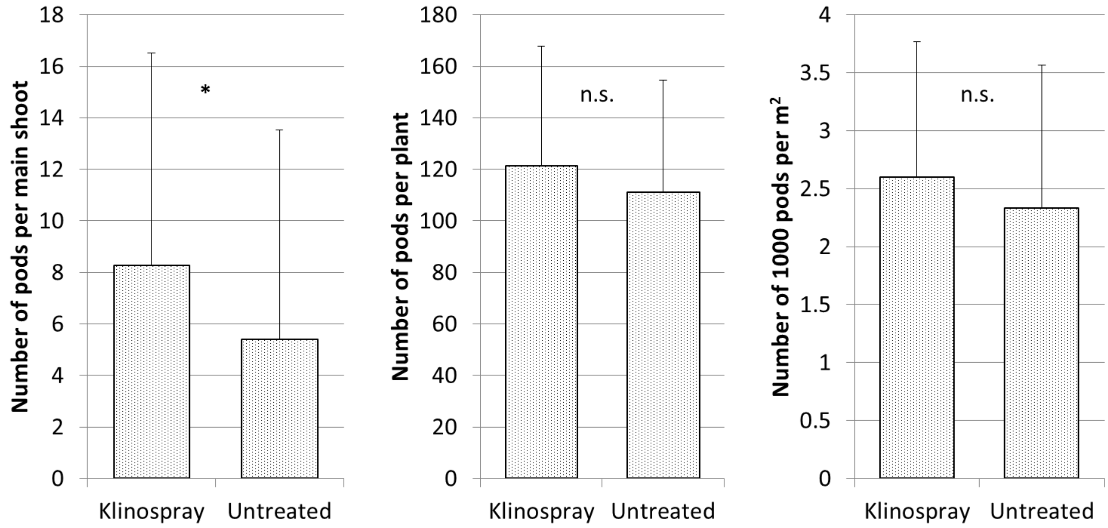

Figure 4. Effect of Klinospray applications on the number of pods per main shoot, per plant and per m^2 in 2010. Statistics: Two-way ANOVA [treatment, field]; main shoot: $F_{1,6} = 6.3$, $p = 0.04$; plant: $F_{1,6} = 0.9$, $p = 0.4$; m^2: $F_{1,6} = 3.9$, $p = 0.09$.

Discussion

Efficacy of Clinoptilolite against pollen beetles ranged between 50 and 80% under dry weather conditions. Under the rainless conditions of 2009, Clinoptilolite provided good control (75% efficacy) throughout the crucial period of blossom development (growth stage 52-58 BBCH). No reduction of pollen beetles was observed under rainy weather conditions. Infestation levels differed substantially between experimental fields and years leading to large standard deviations. Nevertheless, efficacy was similar under high and low infestation pressure.

Efficacy during early stages of blossom development (BBCH 51-52) seemed to be higher than after elongation of the main inflorescence. This observation might be due to two reasons: Coverage of buds with Clinoptilolite and therefore exposure of pollen beetles to Clinoptilolite was much higher after an application at stage 51 BBCH than after an application at stage 55 BBCH. In addition Clinoptilolite, which is used as a deodorizer in cattle production, might have masked the typical odour of the oilseed rape plants and thus disrupt olfactory cues used to locate oilseed rape fields by migrating pollen beetles. This possible mode of action should be further investigated in olfactometer experiments. Moreover, Ebling (1971) showed that inert dusts also have a repellent effect on insects.

The mode of action of Clinoptilolite against pollen beetle is not absolutely clear. Observations of a higher efficacy under dry weather conditions indicate that Clinoptilolite – similar to other sorptive dusts (Mucha-Pezer *et al.*, 2008) might lead to a destruction of the protective wax layer and thus death of the insect by desiccation. Ebling (1971) assumes that desiccation of insects after removal of the protective wax layer is slower at higher relative humidity and that insects can replenish their water loss in moist conditions. However, pore diameter in Clinoptilolite of 4 Å is considered to be too small for absorption of wax molecules. According to Ebling (1971) a pore diameter of at least 20 Å is necessary to absorb wax. In addition Ulrichs *et al.* (2006) showed in laboratory experiments on *Sitophilus granarius*, that mode of action of another zeolite product (Y-Zeolite) is not by desiccation.

In our experiments, dust applications seem to result in a higher efficacy than spray applications. This might be due to two reasons: (1) the lower application rates used in spray applications or (2) the saturation of the product with water and oil from the wetting agent. This again indicates wax absorption as possible mode of action; Ebling & Wagner (1959) showed that spray applications of sorptive dusts had a lower efficacy than dust applications.

The observation that pollen beetles in the Klinofeed treated plots were visibly less active than beetles in the control plots is in accordance with Ebling & Wagner (1959), who noted that treated insects were not as active as the untreated ones, indicating that the presence of dust may have adverse effects aside from that resulting from desiccation.

Three treatments of Klinospray at a rate of 30 kg/ha will cost 375 Euro (Klinospray: 50 Euro/25 kg; Heliosol: 15 Euro/l; labour and machine costs: 35 Euro/application). Thus, to be economically feasible, a yield increase of 230 kg/ha (in organic production; Swiss farm gate prize for organic oilseed rape: 16.5 Euro/t) and 470 kg/ha (in IPM production: farm gate prize: 8.0 Euro/t) would be necessary. The experiments showed that under IPM-conditions a yield increase of 230 to 350 kg/ha is possible. However, yields of 840 kg/ha, as obtained after treatments under severe pollen beetle pressure in 2010, are still far below farmer’s expectations. No yield increase was observed under organic conditions.

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