Influence of synthetic Sitophilate, the aggregation pheromone of *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) on dispersion and aggregation behaviour of the granary weevil

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Abstract

Sitophilus granarius was successfully lured to synthetic Sitophilate, the aggregation pheromone of the granary weevil. Pheromone amounts of 570, 5700 and 57000 ng were tested on groups of unmated males, unmated females, and weevils of mixed sex. Unmated females were more sensitive than unmated males and they were attracted by the pheromone at low dosages. Aggregation of males in the presence of low pheromone dosages was increased when physical contact with females was possible. Very high pheromone concentrations repelled both sexes. The aggregation pheromone is expressed more as an arrestant than an attractant chemical, since weevils stopped moving in sufficiently high pheromone concentrations. The source of the pheromone is not necessarily located. The possibility of using baited traps in the field is discussed.

Introduction

The movement of *Sitophilus granarius* through grain has very often been described as random. Dispersion of the adult weevils occurs mainly horizontally on the surface or in the upper layers of the grain (Surtees 1963, 1964a, 1964b). Although the granary weevil tends to be positive geotactic in undisturbed bulks of grain, its vertical distribution is somehow restricted (Rodionov 1938; Howe 1951). The increasing weight and pressure of grain and the decreasing inter kernel space in the depth of a bulk demand a lot more power and energy from the weevils for pushing their body forward. Therefore their moving ability in deeper layers is limited. Other external stimuli influence weevil behaviour as well. The reaction of the granary weevils toward light has been recorded as negative (Andersen 1938; Torne 1941; Perttunen 1972). Its ability to monitor vibrations with chordotonal organs in the legs was mentioned by Frings and Lollis (1971) but communication and orientation by airborne sounds can be excluded (Wojcik 1969).

Olfactoric sensing of food odours (Donat 1970; Navrot and Czaplicki 1979; Levinson and Kanaujia 1982; Seifelnasr 1991) and other chemical volatiles is by far the most important orientation parameter and vital for finding new food sources and potential mates for the granary weevil. Surtees (1965) did not mention any chemical communication between weevils moving through grain randomly. Faustini et al. (1982) proved the existence of a male produced aggregation pheromone attracting both sexes to suitable sites for feeding, mating and breeding. The chemical identification of the attractant was carried out by Phillips et al. (1987). The aggregation pheromone was named Sitophilate. Synthesised 1-ethylpropyl-2-methyl-3-hydroxypentanoate in the active config-uration of 2S,3R caused attraction in the range of 20 ng to 2500 ng (Phillips et al. 1989). Levinson et al. (1990) recorded receptor potentials even when applying up to 10000 ng of pheromone directly onto the antenna of the weevil. Nevertheless, neither experiments were carried out under more or less natural conditions in the presence of food or in a larger scale of test arena. The current studies were therefore undertaken in order to test the active concentration range of synthetic Sitophilate and the reaction quality of the responding weevils in the presence of wheat. Differences in the behaviour of unmated males and unmated females as well as the interaction between the sexes in the presence of the pheromone lure and the suitable diet for feeding and breeding were recorded.

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Materials and Methods

Weevils

A field strain of *S. granarius* collected from a granary of a river port in Berlin (Germany) in 1988 was reared and multiplied in the Institute for Stored Product Protection of the Federal Biological Research Centre for Agriculture and Forestry under constant conditions of 25°±2°C and 75%±5% r.h. in complete darkness. The parental generation of the test insects was kept on 150 cm^3 wheat of 14% moisture content in 300 cm^3 glass jars for a mating and oviposition period of 14 days. Freshly emerging adults of the F_1-generation were sieved off every other day, isolated by sex and transferred onto fresh wheat. Criteria for sexing the weevils were taken from Halstead (1963). Seven to 10 days old weevils, still unmated but the colour of their cuticula had changed from light brown to dark brown or black, were taken for experiments. Mated weevils used in the experiments had also been sieved off and transferred onto fresh wheat after emerging but were not separated by sex.

Pheromone

One mL pure (2S,3R)-1-Ethylpropyl-2-methyl-3-hydroxypentanoate with the pheromone concentration of 9.5 µg/µL was donated from the Department of Entomology from the University of Wisconsin in Madison, WI (USA).

Three diluted fractions of 0.95 µg/µL, 0.095 µg/µL and 0.0095 µg/µL were drawn from the original sample by using n-hexane as diluting agent. By applying 60 µL of a fraction on a rubber septum the actual pheromone amounts of 570 ng, 5 700 ng and 57000 ng were offered to the weevils. 60 µL of n-hexane were applied on a rubber septum in the untreated control experiments.

Biotest

A box of perspex glass, 30 cm in square by height 5 cm was filled up to 3.5 cm with wheat of 14% moisture content. The total grain volume was ca. 3000 cm^3. It was possible to quickly insert a grid also made out of plastic into the box to divide the grain volume into 25 equal smaller parts or fields which were numbered in order (see Fig. 1). The pheromone lure was placed in field IV/4. The test insects were released in field III/2 after a 10 min adaptation period. The top of the box was covered with a plate of glass.

The insects were allowed to move around in the arena freely, to feed and rest at places of their choice. After 24 hours, the box was opened and the grid inserted quickly. Each field was sucked out with a vacuum pump and checked for weevils. By this, the present abode of every weevil after an exposure time of 24 hours to the pheromone was recorded.

The three different pheromone concentrations with the actual amounts of Sitophilate were tested on groups of 25 weevils. Unmated males and unmated females as well as groups of 13 mated males and 12 mated females were tested separately. The lures were usually tested for 28 days, and for 14 days on groups of mixed sex. All test series were carried out in three replicates under constant conditions of 25°±2°C and 75%±5% r.h. in darkness. When releasing the insects and during emptying the fields, only red light was switched on.

Mathematical analyses

After recording the present abode of every weevil after 24 hours an 'Aggregation Value' (AV) was calculated. This Aggregation Value described the distribution of the weevils in the system by considering the number and the intensity of possible occurring aggregations. The intensity of one or more aggregation spots depended upon the amount of weevils occurring in clusters. The Aggregation Value was calculated by the quotient of the 'Mean Distribution' (MD) and the 'Distribution Index' (DX).

MD represented the average number of individual weevils occurring together and forming clusters. The maximum value for MD was 25, when all individuals appeared in one single field. The minimum value was 1, when every weevil was found alone in one field.

DX described the overall distribution and was the average number of fields being occupied by at least one weevil in relation to the total number of available fields. The maximum value was 1, when all 25 field were occupied by one weevil each. The minimum value was 0.04, when all weevils occurred together in one field.

Therefore the AV showed possible values between 625 for complete aggregation of all weevils at one spot, and 1 for maximum dispersion, when the weevils appeared the furthest apart from each other.

Missing in the Aggregation Value was the quality of the aggregation, since an aggregation in the test arena could have been caused by attractive or repellent effects of the lure. Therefore, the test arena was divided into four zones (see Fig. 2). Zone A consisted of field IV/4, containing the pheromone lure. Zone B covered the eight fields surrounding zone A. Weevils recorded in these two zones had to move at least once towards the lure. So, the reaction of these weevils was regarded as positive, the effect of the pheromone as an attractive one. Weevils being recorded in the outer zone D of the arena moved at least once away from the lure. Their reaction was therefore stated as negative and the effect of the pheromone as a repellent one. Zone C was called a neutral zone because weevils walking around in this area were able to move through the grain without necessary turning towards or away from the pheromone source. The number of negative reacting weevils was subtracted from the number of weevils showing positive reactions and divided by the total number of insects in the system (Busvine 1971). The reaction quality was estimated simply by setting the data of the untreated control, which had been calculated the same way, as zero. Positive values stand for an attractive effect, negative values for a repellent effect of the lure.

The overall distribution was shown by adding up the relative accumulations of the weevils in the different zones. The actual distances of the present abode of the weevils to the pheromone lure were compared to the general distribution pattern in the untreated controls.

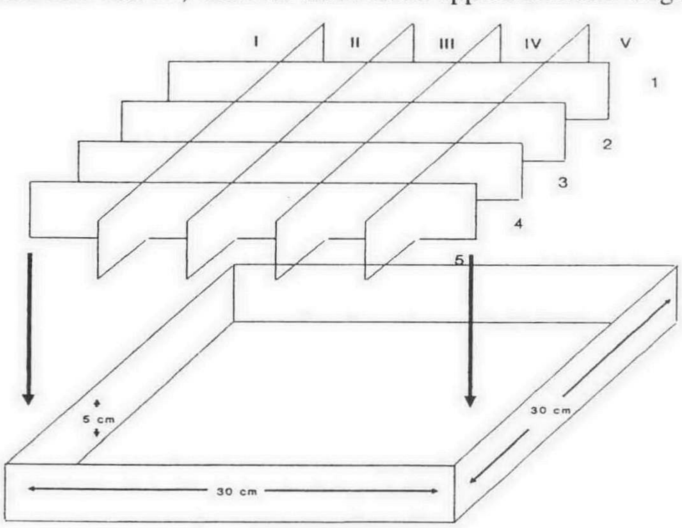

Fig. 1. Test arena for biotests (unfilled). A grid could be inserted quickly to divide the grain into 25 equal parts (fields). The parts were numbered in order from I/1 to V/5.

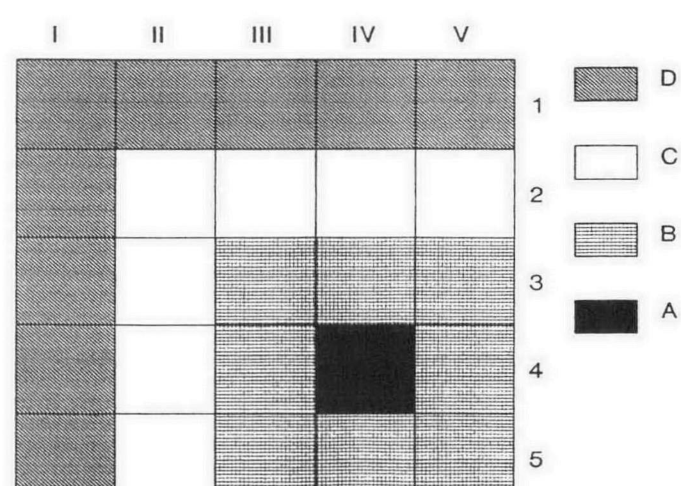

Fig. 2. Test arena for biotests (viewed from above) showing the different zones A to D for qualifying weevil behaviour towards or away from the pheromone lure.

Table 1. Distribution of granary weevils *S. granarius* in the untreated control

	Unmated males	Unmated females	Mixed sex
DX value	0.48±0.07	0.55±0.04	0.55±0.05
MD value	3.03±0.43	2.28±0.21	3.21±0.45
AV value	6.31(6.34/6.29)[a]	4.15(4.22/4.06)[a]	5.84(6.10/5.52)[a]
Distribution			
Zone A	1.33%±0.95%	1.33%±0.95%	2.31%±0.95%
Zone B	22.67%±3.43%	17.33%±1.90%	30.67%±1.90%
Zone C	33.33%±7.43%	22.67%±1.90%	25.33%±2.52%
Zone D	42.67%±4.33%	58.67%±3.43%	41.33%±1.90%

[a]Reliable range of the AV value, being calculated from lower and upper range of DX and MD values.

Graphical display

The results of all the tested pheromone concentrations for the different groups of test insects were presented in five graphs (a to e) each. Graphs a to c show the calculated DX-, MD- and AV-values over the time of 28 days. The solid horizontal line in every graph represented the mean value in the untreated control, the dotted lines its reliable range. Graph d showed the attractive or repellent quality in the reaction of the weevils. Graph e represented the relative accumulation of the insects in the different zones of the arena. The horizontal solid lines represented the distribution in the untreated control (lower line for zone A and B together, upper line for zone D).

Results

Untreated control

In the untreated controls (see Table 1) unmated males show a higher Aggregation Value than unmated females. The DX-value is lower for unmated males indicating a less wide distribution in the arena. Unmated females instead occupy on average over 50% of the available space. It can be seen from the MD-value that, on average, more unmated males appear together in one field than unmated females do.

The distribution patterns show that, on average, one-fourth of the unmated males appears in zones A and B whereas one-fifth of the unmated females occupies this area. The outer zone D shows mean distribution values of over 40% for unmated males and nearly 60% for unmated females. The corners and the periphery of the box are evenly occupied but slightly preferred over the middle of the arena.

Groups of mixed sex (13 males and 12 females) occupy, like unmated females, on average half of the available space in the arena. With a slightly higher MD-value weevils in groups of mixed sex form on average more intense clusters than unmated male weevils did. But weevils in groups of mixed sex occur in fewer aggregations. Their Aggregation Value becomes lower than the one for unmated males. The distribution patterns indicate an even distribution in the combined zone of A and B and in zone D.

Effects of the pheromone on unmated males

When 570 ng of pheromone was applied onto the rubber septa and offered to unmated males, no clear effect is visible when compared to the untreated control. The DX- (see Fig. 3a) and the MD- (see Fig. 3b) Values with their reliable ranges do not differ from the control. The calculated AV-value (see Fig. 3c) oscillates around the control value showing no obvious tendency. Regarding the reaction quality (see Fig. 3d) and the distribution pattern (see Fig. 3e), only a very small and insignificant repellent effect of the lure is visible.

The application of 5700 ng of Sitophilate leads to a slightly different behaviour pattern in unmated males (see Fig. 5a to 5e). The Aggregation Values indicate that up to the 8th day after pheromone application a wider distribution of the weevils occurs than in the untreated control. The weevils do not aggregate as much as they do in the absence of the lure. The reaction quality shows positive responses of the unmated male weevils in the first 3 days and again for a shorter period after 9 to 11 days. This indicates an attractive effect of the lure. The distribution pattern, where more than 40% of the unmated male weevils appear in closer range to the pheromone source on day 3 and on day 11 verify the observation. After the 12th day, the behaviours of the weevils become very much comparable to those in the presence of the 570 ng pheromone lure.

Regarding the responses of unmated male weevils to the high dosage of 57000 ng of pheromone, a clear repellent effect of the lure is visible (see Fig. 7d). Nevertheless, the weevils aggregate in the first 9 to 13 days (see Fig. 7c) to a very high extent. The aggregation occurs in zone D, the far side of the pheromone (see Fig. 7e). Fourteen days after pheromone application, the distribution pattern slowly shifts towards the situation of the untreated control.

Effects of the pheromone on unmated females

Pheromone amounts between 570 ng and 57000 ng cause an aggregation of unmated females in the first 8 to 10 days after pheromone application (see Fig. 4c, 6c, 8c). The reaction quality is strongly positive in the presence of 570 ng and 5700 ng of Sitophilate. Still showing a positive tendency, the reaction values oscillate very much around zero in the presence of 57000 ng. Unmated female weevils do not move very far from the point of release when the highest pheromone dosage is applied.

In the presence of 570 ng of pheromone 20% of all unmated females are attracted into zone A, the very close space around the lure (see Fig. 4e). Fifty per cent and more unmated females occur in zone A and B together during the first 5 days after application. Compared to the untreated control, twice as many unmated female weevils stay in that area for the first 13 days.

With increasing amount of pheromone being offered, the percentage of unmated females being recruited into zone A decreases (see Fig. 6e, 8e). The weevils react positively towards the lure but do not continue the movement all the way to the pheromone source. With passage of time the distribution pattern shift to the distribution situation in the untreated control.

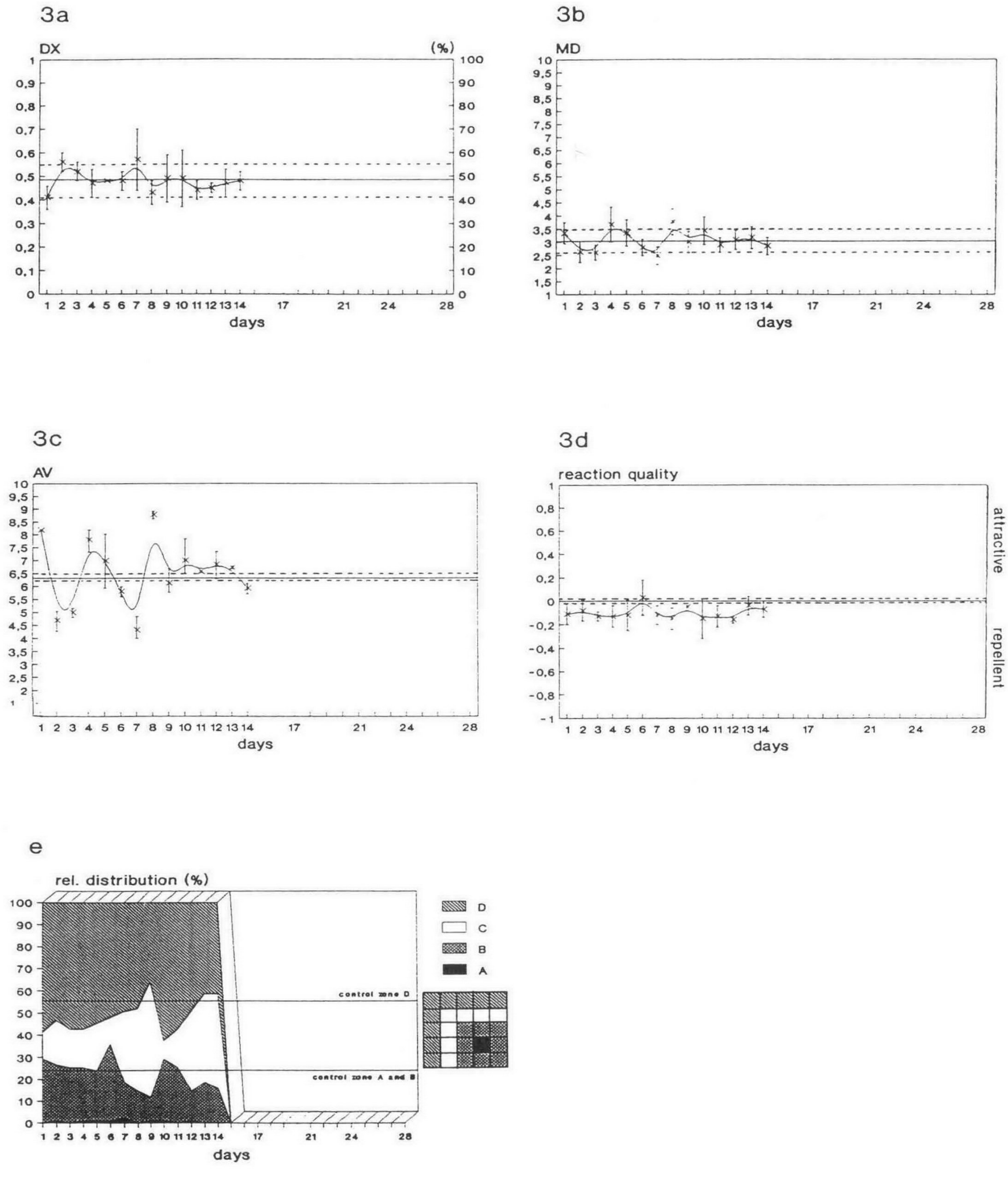

Fig. 3. Distribution behaviour of unmated males in the presence of 570 ng of Sitophilate (a) distribution index (DX-value), (b) mean distribution (MD-value), (c) aggregation value (AV-value), (d) reaction quality and (e) distribution pattern.

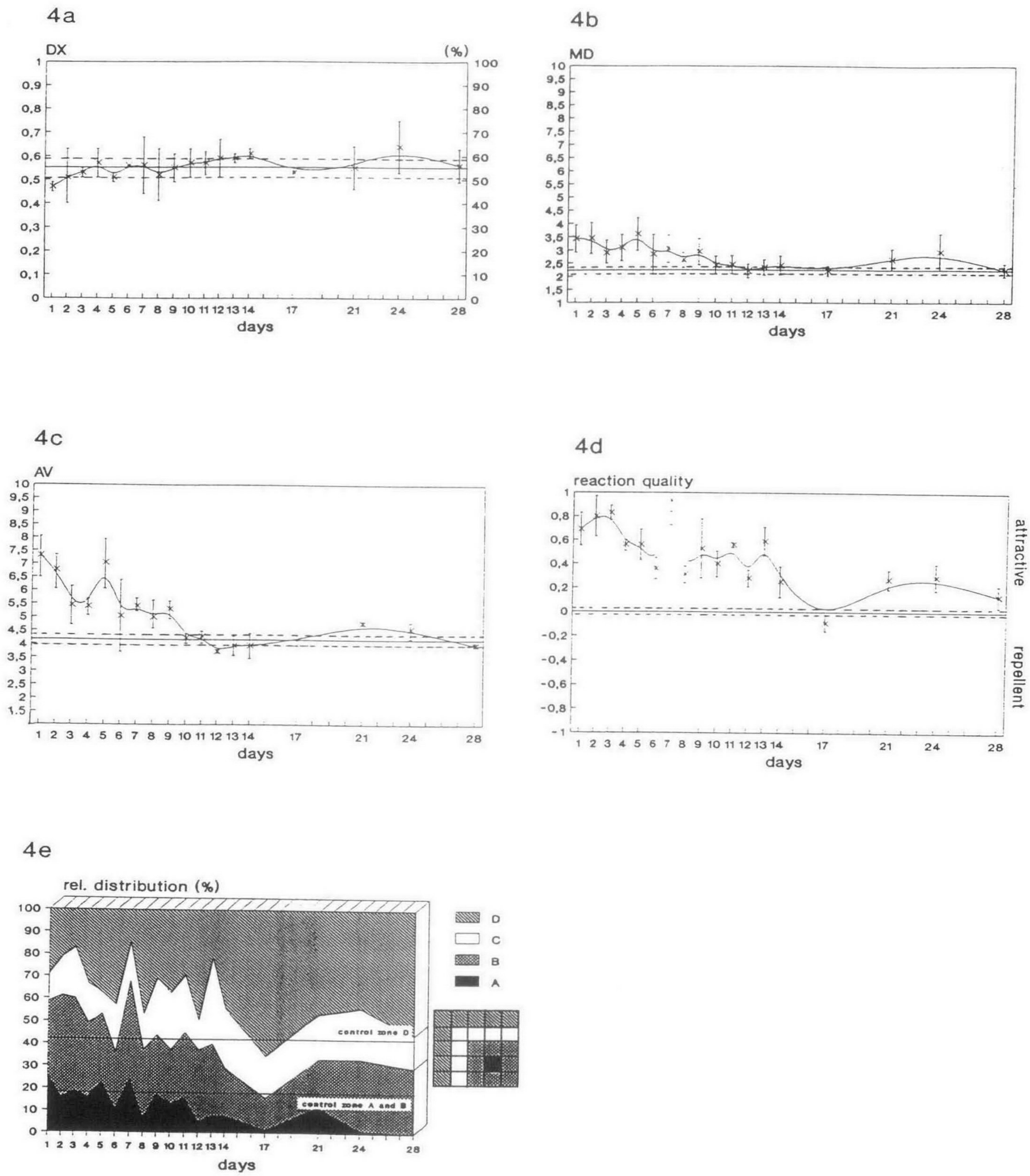

Fig. 4. Distribution behaviour of unmated females in the presence of 570 ng of Sitophilate (a) distribution index (DX-value), (b) mean distribution (MD-value), (c) aggregation value (AV-value), (d) reaction quality and (e) distribution pattern.

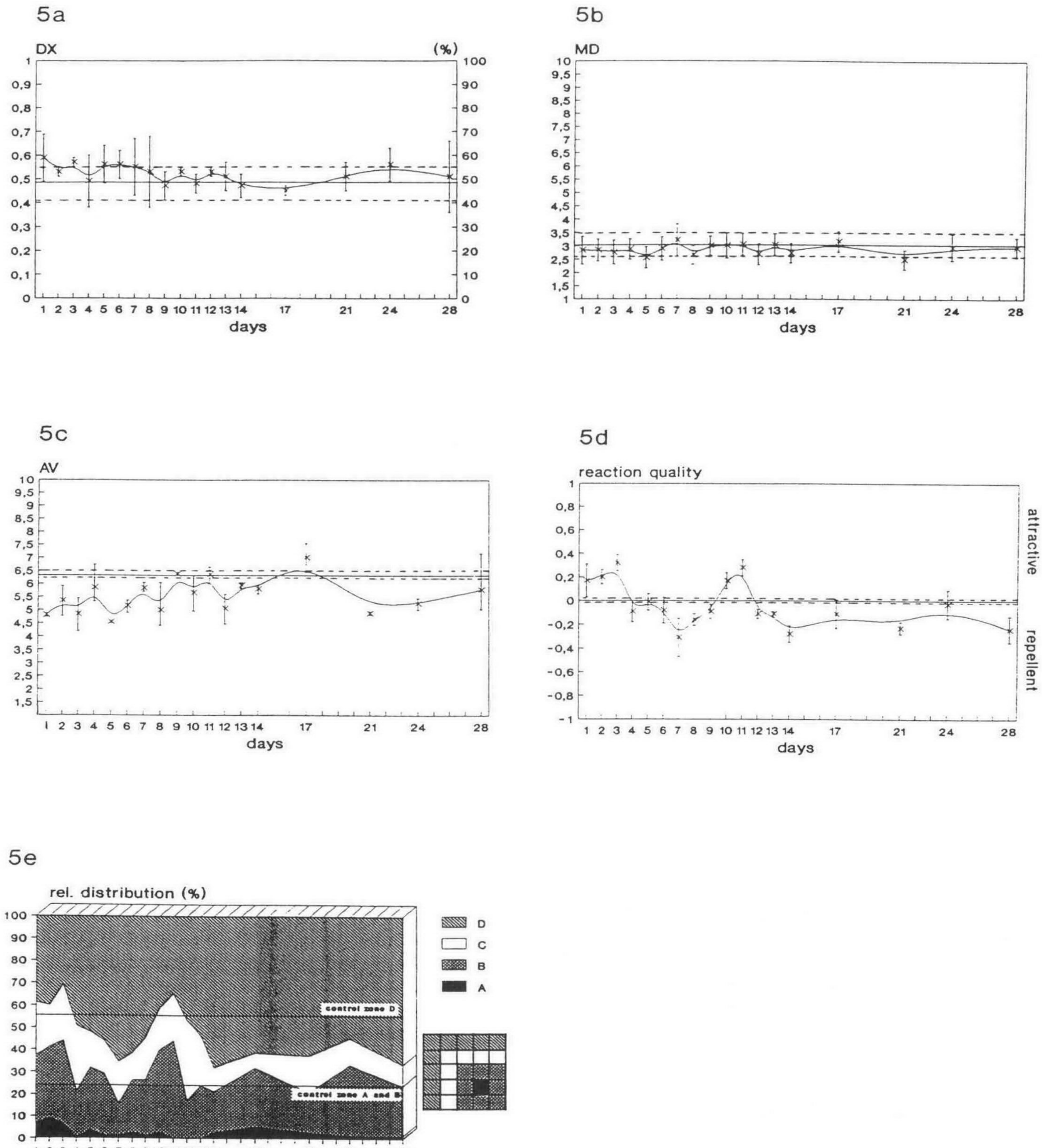

Fig. 5. Distribution behaviour of unmated males in the presence of 5700 ng of Sitophilate (a) distribution index (DX-value), (b) mean distribution (MD-value), (c) aggregation value (AV-value), (d) reaction quality and (e) distribution pattern.

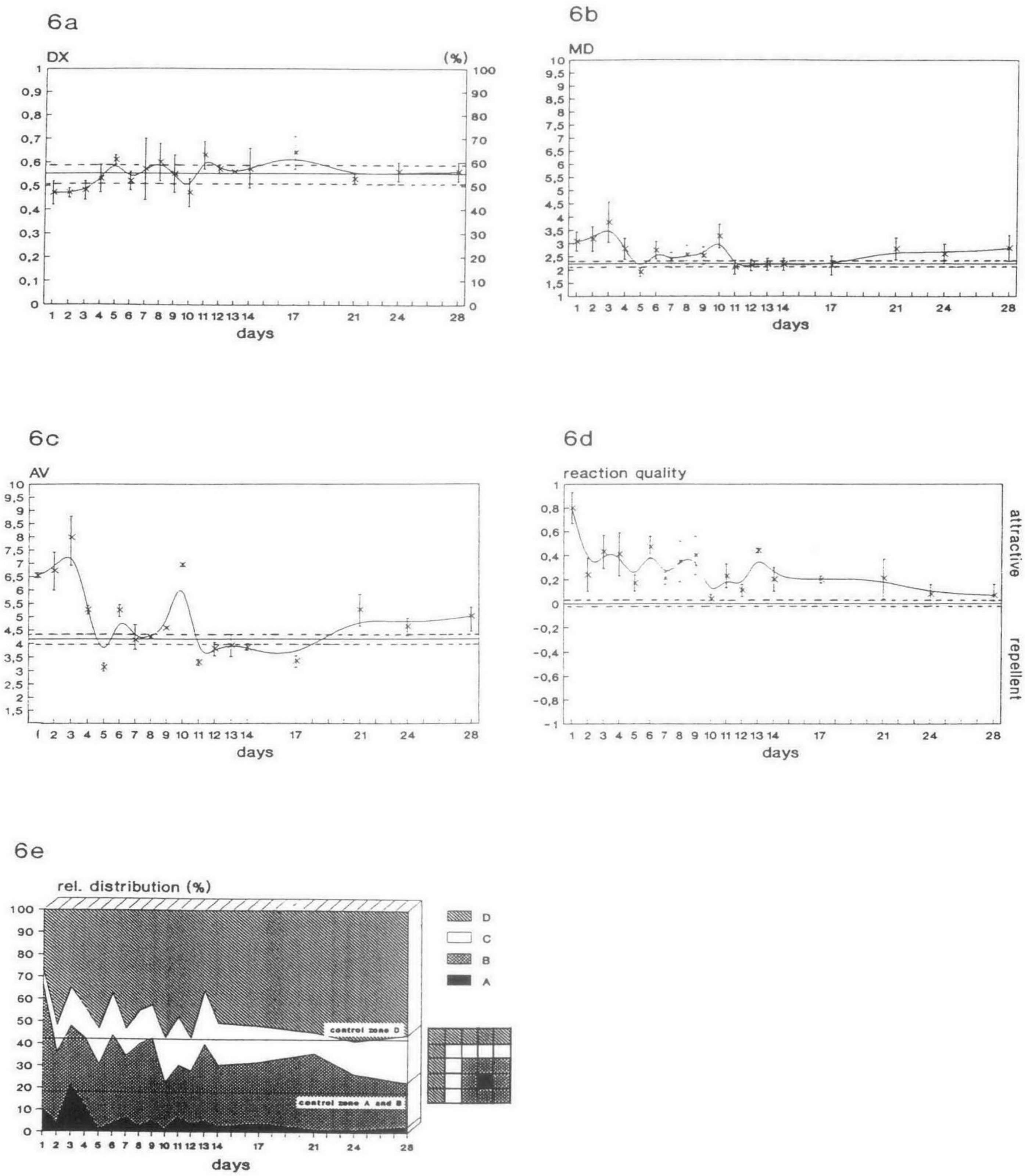

Fig. 6. Distribution behaviour of unmated females in the presence of 5700 ng of Sitophilate (a) distribution index (DX-value), (b) mean distribution (MD-value), (c) aggregation value (AV-value), (d) reaction quality and (e) distribution pattern.

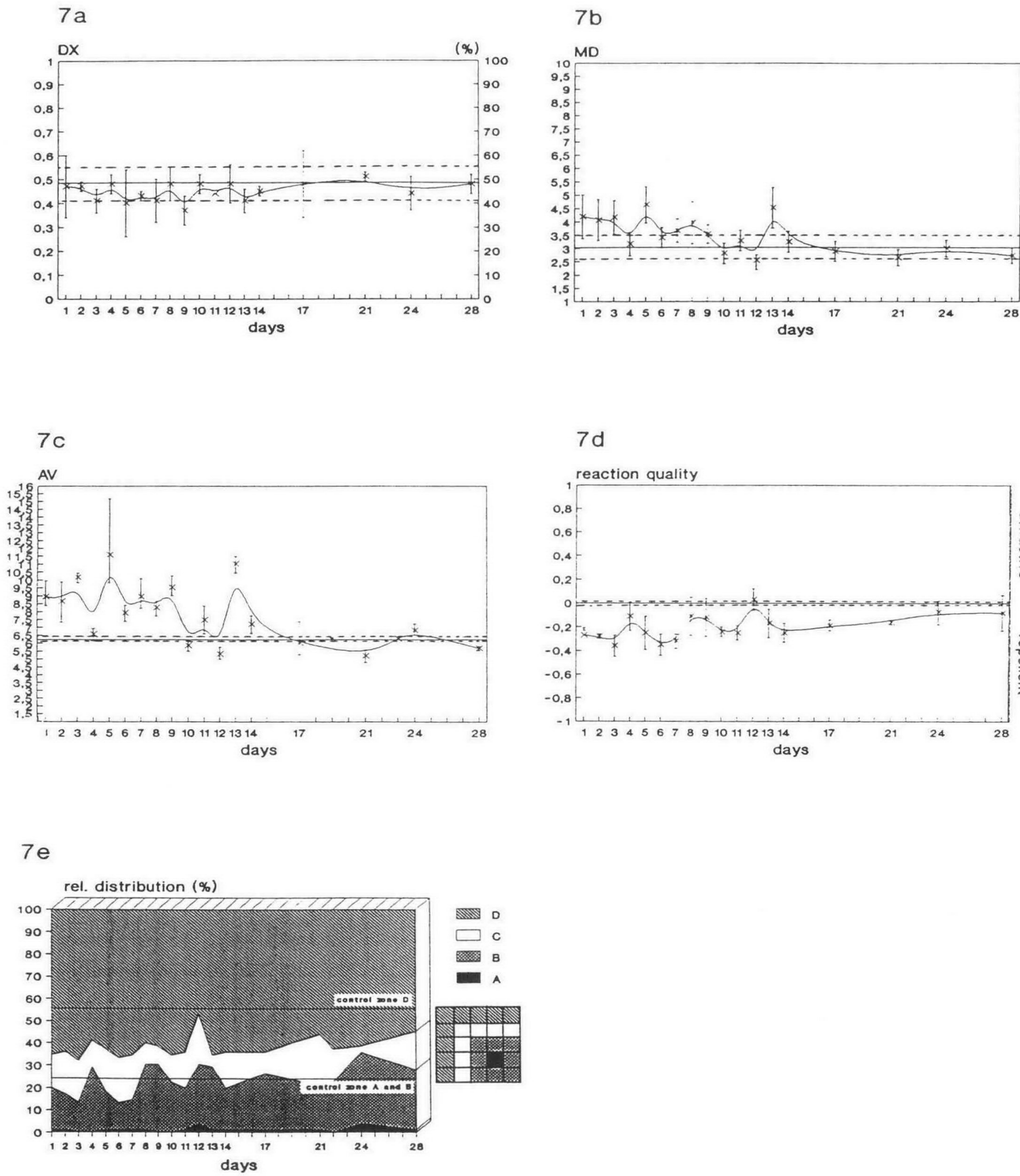

Fig. 7. Distribution behaviour of unmated males in the presence of 57000 ng of Sitophilate (a) distribution index (DX-value), (b) mean distribution (MD-value), (c) aggregation value (AV-value), (d) reaction quality and (e) distribution pattern.

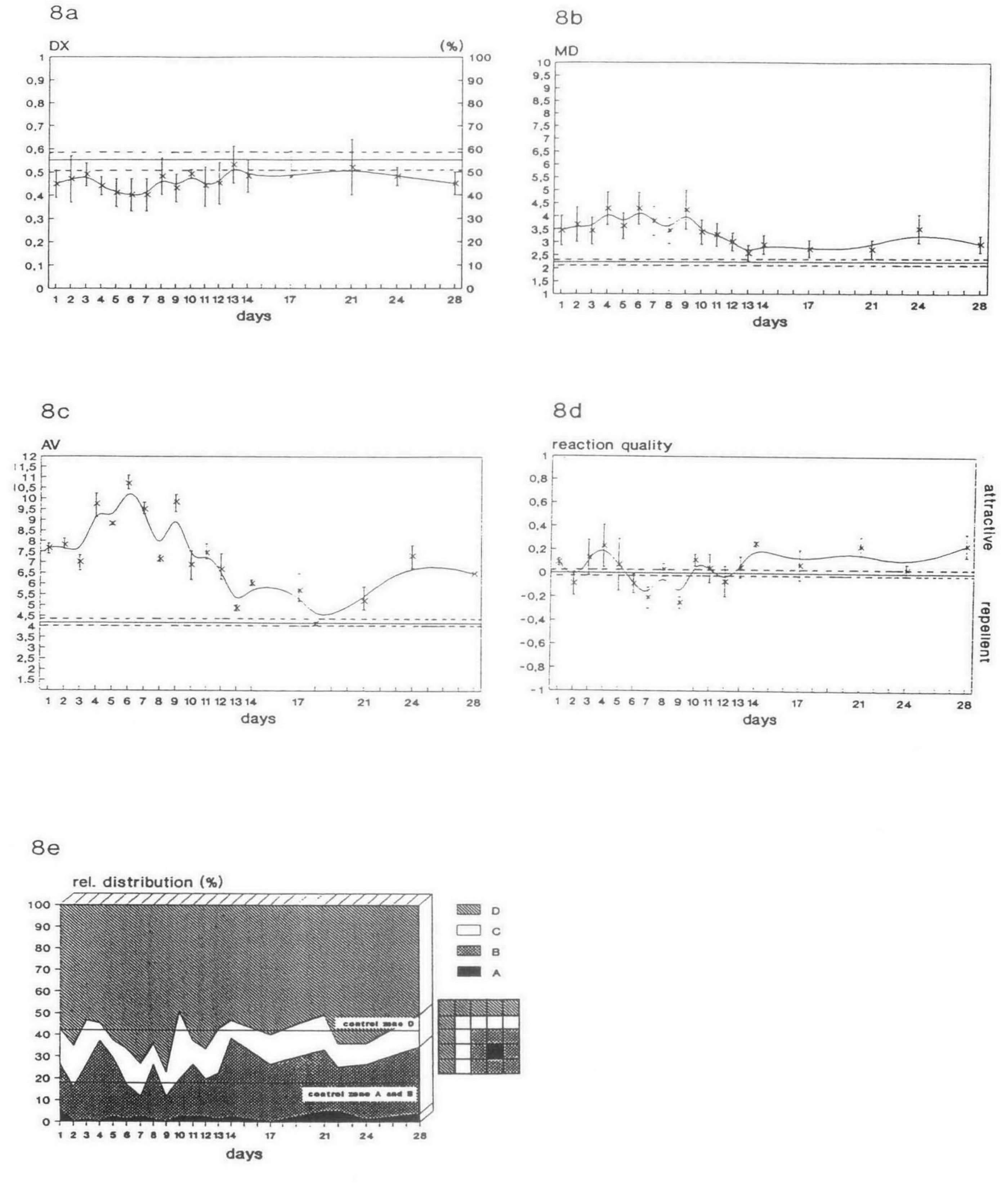

Fig. 8. Distribution behaviour of unmated females in the presence of 57000 ng of Sitophilate (a) distribution index (DX-value), (b) mean distribution (MD-value), (c) aggregation value (AV-value), (d) reaction quality and (e) distribution pattern.

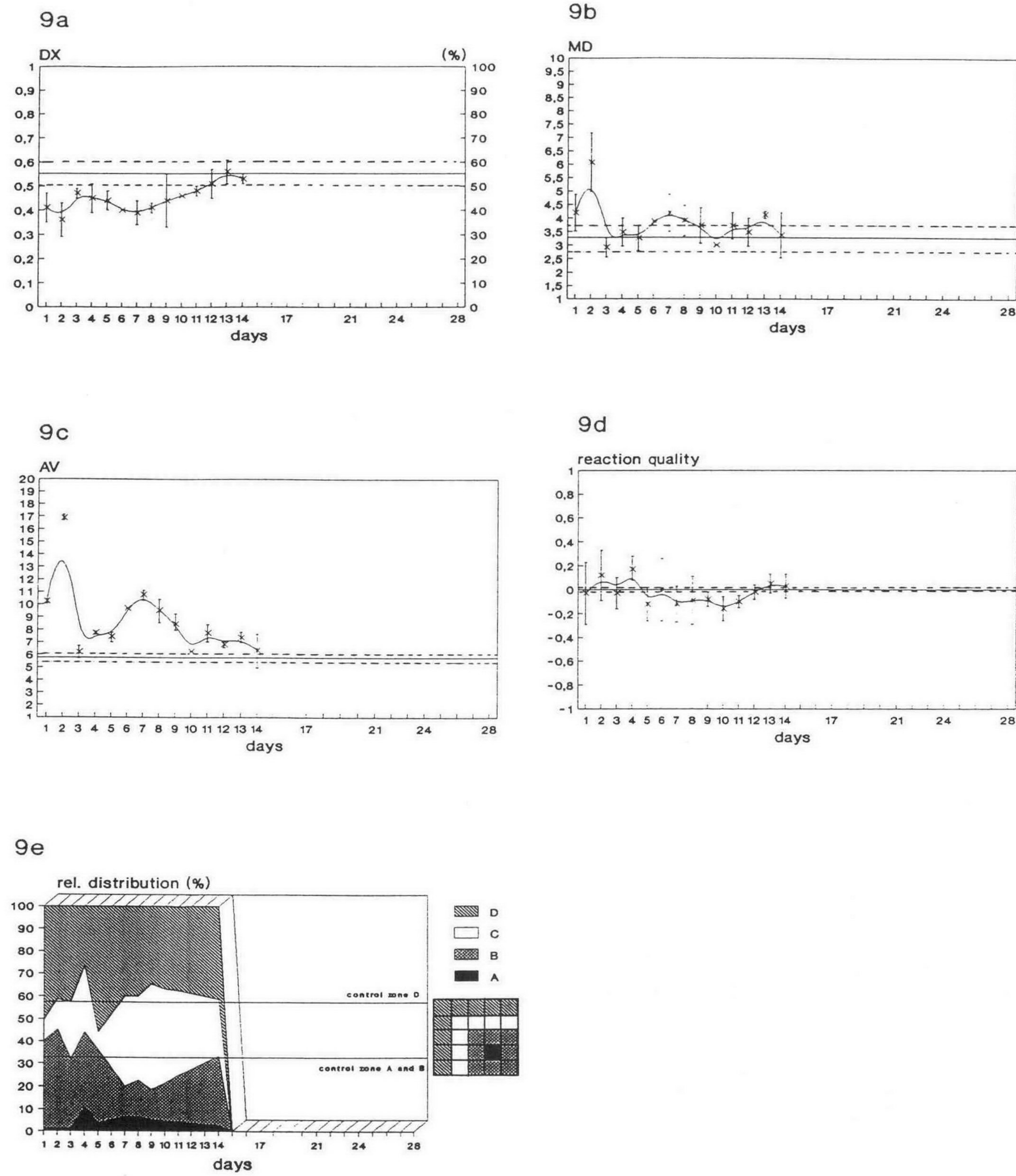

Fig. 9. Distribution behaviour of groups with weevils of mixed sex in the presence of 570 ng of Sitophilate (a) distribution index (DX-value), (b) mean distribution (MD-value), (c) aggregation value (AV-value), (d) reaction quality and (e) distribution pattern.

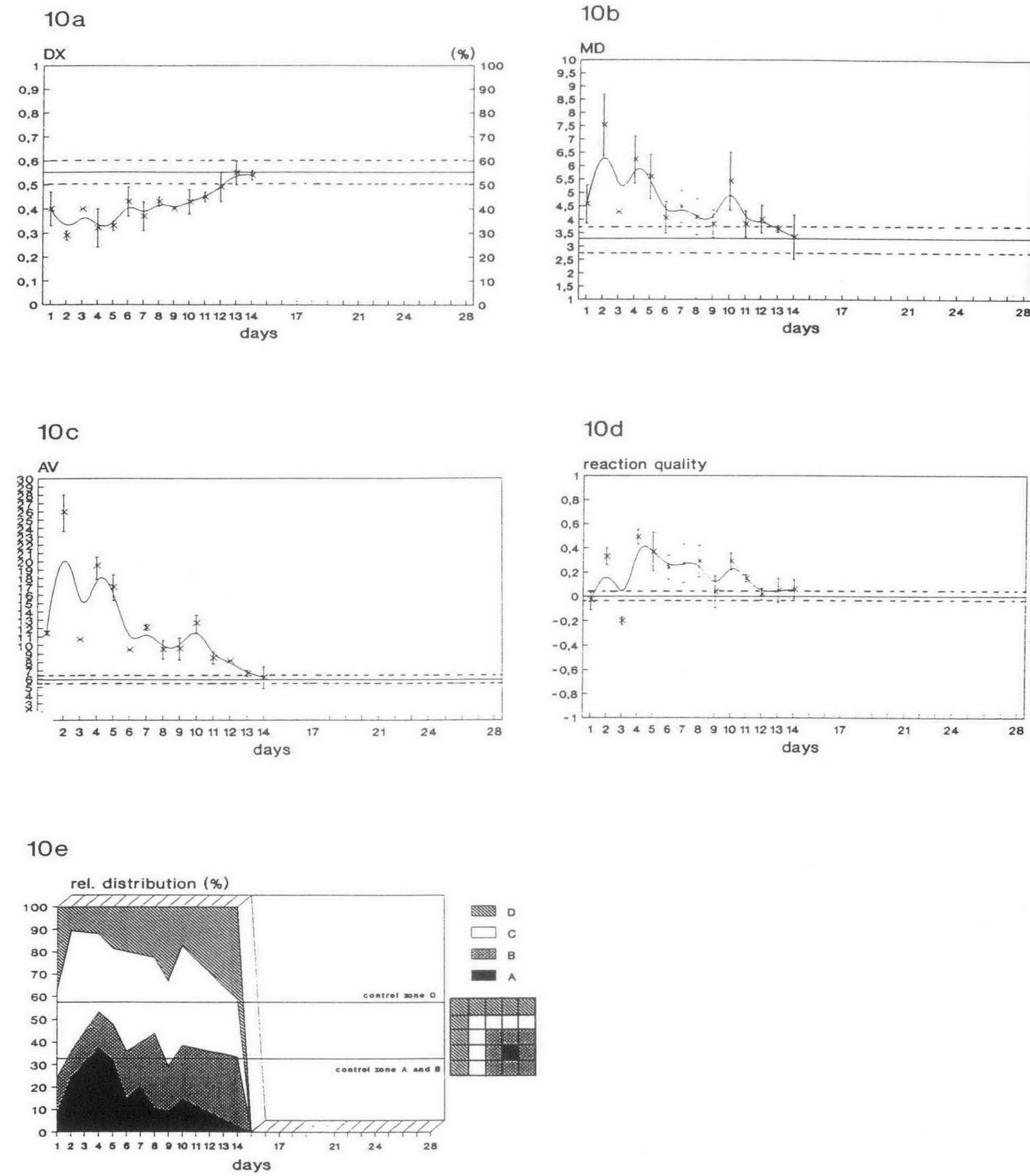

Fig. 10. Distribution behaviour of groups with weevils of mixed sex in the presence of 5700 ng of Sitophilate (a) distribution index (DX-value), (b) mean distribution (MD-value), (c) aggregation value (AV-value), (d) reaction quality and (e) distribution pattern.

Effects of the pheromone on groups of mixed sex

Only 570ng and 5 700 ng of pheromone were offered to weevils in groups of mixed sex (see Figs 9 and 10). Even this small amount of pheromone causes higher aggregations than those which appear in the untreated control (see Fig. 9c). It can be taken from the distribution pattern (see Fig. 9e) that in the beginning the weevils stay close to the point of release. Three days after pheromone application more weevils move towards the pheromone and aggregate close to the emission source. Nevertheless, the majority of the weevils still stay in zone C. After the 9-hour day the distribution pattern matches the situation of the untreated control.

Similar, but more distinct ,is the reaction of the weevils in the presence of 5700 ng of pheromone. After staying and aggregating in the area of release, the weevils show a strong positive reaction towards the pheromone. Aggregation near the pheromone source reaches its height on the 4th day after application. At that time 50% of all weevils are attracted to the zones A and B with over 30% surrounding the actual lure. With the decay of the pheromone over time the distribution pattern meets more and more those of the untreated control.

Discussion

Attraction of *S. granarius* towards synthetic Sitophilate is possible. This has been demonstrated by Phillips et al. (1989). The present study primarily documents the successful luring of granary weevils to a pheromone source in the presence of grain, the granary weevils' natural habitat. Although both sexes, in the virginal as well as in the mated state, reacted positively to the synthetic aggregation pheromone, sex specific behaviour became obvious.

Since the pheromone is male produced, unmated males were able to attract each other and form aggregation spots even in the absence of a pheromone-baited lure. Females are pheromonal inactive, so, unmated females appeared to move rather randomly in the arena when no pheromone was offered.

Unmated females responded with positive reactions when 570 ng of pheromone were presented, and 20% of the unmated female weevils tracked down the source of the chemical. Higher dosages of the pheromone also led to aggregations of unmated females but the aggregation sites occurred further away from the actual lure. This leads to the idea that the aggregation pheromone is not so much an attractant like many sex pheromones but more an arrestant (Evans 1984). The presence of the attractant induces movement, recruiting the individual along a concentration gradient towards the pheromone source. When the weevil reaches a sufficiently high concentration it slows down and eventually stops. This could be the case in the presence of higher pheromone dosages, when unmated females did not continue the walk all the way to the pheromone source. The message of an aggregation pheromone is to bring the individuals of a species together to influence the micro climate by increasing locally the temperature and relative humidity. Physical contact with other weevils might already fulfil the aim of forming an aggregation and therefore could lead to a halt in the movement as well. If that happens it becomes unnecessary to continue to follow the concentration gradient of the luring chemical.

The application of 57000 ng of Sitophilate caused aggregation in unmated females in the far side of the arena away from the lure. This indicated a possible repellent effect of very high amounts of the pheromone. The same was true in unmated males when very high amounts of pheromone were offered. Their reaction quality was apparently negative. The effect of the lure was clearly repellent when 57000 ng were offered.

In contrast to the high amount of pheromone, the low dosage of 570 ng of pheromone caused no difference in distribution behaviour of unmated males when compared to the untreated control. This might be because the pheromone-producing sex is always surrounded by small quantities of the attractant and is therefore unable to sense little amounts of that chemical.

The intermediate dosage of 5700 ng of Sitophilate caused positive reaction in unmated males, and the weevils occurred closer to the pheromone source, but they were not really aggregating like the unmated females did. Either, unmated males were not able to distinguish between the lure and other males emitting pheromone and therefore were somehow disorientated, or the lack of physical contact with females led to a quick disbandment of the clusters in order to continue the search for more suitable aggregation spots. The absence of the opposite sex would therefore result in an increased mobility of male weevils in the area of sufficiently high pheromone concentrations, which normally would attract females as well. This can also explain the accumulation of male weevils when tested in groups of mixed sex, where males and females formed aggregations to a great extent when 5700 ng of pheromone were present. In many of these aggregation spots males and females occurred together in equal numbers.

There is still a long way to go before using pheromone-baited traps for monitoring or even mass-trapping of *S. granarius* in the field. Tests with pheromone-baited probe traps in larger scales of grain have been carried out recently and are quite promising (R. Plarre unpublished data) for monitoring purposes. Similar statements were made for *S. zeamais* by Walgenbach et al. (1987). It is important to apply only the optic pure pheromone, but synthesis of this chemical is still expensive. Sitophilate is very unstable and degrades quickly. Perhaps rubber septa are not the best means of dispensing pheromone. Having to change the lure every week is not economically justifiable. The amount of pheromone being emitted from a lure is also very important, since concentrations which are too high stop the movement of the *S. granarius* towards the trap, or even repell them.

Acknowledgments

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