Storage arthropod pest detection – current status and future directions

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Abstract

A key element of a grain storage integrated pest management (IPM) system is the ability to monitor for the presence of arthropod pests and to reliably detect these, as early as possible, at low population densities. There has been considerable progress in the development of traps for monitoring storage insects and mites and progress has also been made in the development and validation of attractant lures to augment the traps and improve sensitivity. Proper interpretation of trap catch is an area for which further research is needed and this is linked to a requirement for risk assessment to enable appropriate prevention or control strategies to be implemented, based on pest presence. Improved knowledge of arthropod biology and behaviour is key to this area. This paper examines the latest progress in the development of attractant lures for insect traps, in particular the development of a multi-species lure for stored-product insects. It also reports on findings from recent research on aspects of arthropod biology and behaviour to improve understanding of the factors and interactions that influence trap catch. This includes studies on insect mobility and the response to temperature and moisture gradients. Finally, it will consider the research required to provide proper interpretation of trap catch and the integration with risk assessment strategies.

Key words: Detection and monitoring; Multi-species lure; Behaviour; Stored product insects and mites; Risk assessment.

Introduction

Stored grain is at risk to infestation by a range of stored-product insects and mites. There are significant economic costs associated with insect infestation, not only in terms of direct damage to the commodity, but also in terms of the costs associated with rejection. Effective detection of pests is an essential tool when protecting harvested crops; revealing whether control is necessary and whether, once implemented, it has been successful. Monitoring for the presence of beetle and mite pests is therefore a key requirement of IPM strategies for stored grain. Over the past few decades a range of traps and monitoring devices have been designed and validated for monitoring of insect presence, in the commodity itself (e.g. Loschiavo and Atkinson, 1967; Cogan et al., 1990) and associated buildings (Wyatt et al., 1989; Mullen, 1992; Collins and Chambers, 2003). However, research has shown that these monitoring devices still only detect a small percentage of the arthropod pests present. There is therefore a need to increase the sensitivity of these traps and monitors. This can be achieved by the addition of an attractant lure. Aggregation pheromones, which act as attractants, have been identified for the principal storage beetles (see review by Plarre and Vanderwal, 1999). In general, these will attract only one species, consist of more than one compound and are difficult to synthesise on a cost-effective basis. The use of a lure based on food attractants holds promise as a basis for a multi-species lure and allows for expansion of the number of target species to include those for which the attractant pheromones are unknown. It also allows...
for detection of larval stages. However, food attractants are complex and less well understood than pheromones (Chambers, 2002). Research on the behavioural effects of food volatiles on stored-product pest insects was reviewed by Cox and Collins (2002) and a food attractant for the foreign grain beetle, *Ahasverus advena* (Waltl.), has been simplified recently (Wakefield et al., 2005).

In addition to improving the sensitivity of traps, information is required on the interpretation of trap catch. There is a need to correlate numbers of insects found with numbers likely to be present and to use this information to assess control measures that may be necessary. In order for this to be achieved effectively, knowledge of the biology and behaviour of the target species is required. Recent research at the Central Science Laboratory has examined the effect of physical gradients of temperature and moisture on insect movement. The minimum temperatures for insect movement, both adult and larval, and for flight for a range of species have also been established. This information will enable effective strategies for deployment of traps to be implemented and will assist in interpretation of trap catch. Further research is required to fully understand insect behaviour in response to volatile cues and traps. Recent EU legislation encouraging adoption of HACCP (Hazard Analysis Critical Control Point) approaches for feed hygiene (EC No. 183/2005) demands that methods to identify the presence and level of risk, in this case arthropod infestation, are put in place. Arthropod monitoring and correct interpretation is therefore of great importance. This paper describes recent research at the Central Science Laboratory to optimise insect and mite monitors and studies of behaviour that will affect trap catch. It also considers the future research necessary for effective interpretation of trap catch in monitoring programs.

**Multi-species lure (beetles)**

The potential of food derived volatiles as multi-species attractants has long been recognised and one of the first monitoring devices for crawling insects, the bait bag, made use of the presence of foodstuffs (Strong, 1970). Foodstuffs contain a complex mixture of volatiles, only some of which will be detected by the insect and elicit a behavioural response. To avoid the possibility of infestation of the monitoring device itself, as is possible with the bait bag, researchers have examined the use of extracts of the foodstuffs (Cox and Collins, 2002 and references therein). This can be a time consuming process and owing to the batch-to-batch variation of natural products the final material cannot be produced consistently. The advantages of identifying the key compounds in a complex mixture of food volatiles that are responsible for the attraction are (1) consistency of the material, (2) reduced costs (3) ease of production and (4) elimination of materials that may potentially affect the attractiveness of the material. Ideally the attractant lure would contain only the compounds known to be attractive and these would be easily obtainable so that lures could be produced economically. The production of such a lure has been examined using a mixture of volatiles obtained from kibbled carob and peanuts which had previously been shown to attract the three principal UK storage beetles *Oryzaephilus surinamensis* (L.), *Sitophilus granarius* (L.) and *Cryptolestes ferrugineus* (Stephens) (Wakefield, unpublished data). Electrophysiological studies showed that the three species responded to different compounds within the mixture and for *S. granarius* there were significant differences in the response by males and females. Responses were elicited from less than 10 % of the 180 compounds present for all test insects of a particular species (Collins et al., 2006). Initial behavioural testing of compounds indicated eleven compounds that were of interest for further studies. Of these, only two compounds, E-2-nonenal and 4-ethylacetophenone, elicited a response from all three species. Three compounds, 2-phenylethanol, hexanoic acid and E-3-octen-2-one, elicited a response from two of the three species (Collins et al., 2006). Further studies to optimise the attractant response for all three species resulted in a six component mixture consisting of hexanoic acid, 3-methylbutanol, 4-ethylacetophenone, 3-octen-2-one, nonanal and E-2-nonenal (Wakefield et al., 2006). This six-component mixture was used in laboratory bioassays to determine the range of species for which the mixture was attractive, focussing on key species identified by industry. An
additional four of the eleven species tested showed a significant increase in the number of insects responding to both the six-component mixture and to the carob-peanut extract. Four of the species responded to the carob-peanut extract, but not to the test mixture and three of the additional species tested, did not respond to either the test mixture or to the carob-peanut extract (Table 1).

Further laboratory studies identified a polythene vial as the most suitable dispenser for the volatile mixture and established that the lure was effective for a period of up to six weeks (Wakefield et al., 2006). The performance of this prototype lure in PCTM traps and PC Floor traps was evaluated under more practical conditions both in the Storage Research Facility at CSL and in commercial premises. It was found that significantly more C. ferrugineus were found in PCTM traps containing the lure both on the grain surface and buried below the surface. With O. surinamensis and S. granarius greater numbers of insects were generally found in PCTM traps with the lure, although these differences were not significant. Significantly more O. surinamensis were found in PCTM Floor traps containing the lure compared to those without the lure. It was concluded that in general, the increases in trap catches were modest and the lure was more effective in the PCTM Floor Traps than in the grain bulk (Wakefield et al., 2006). Trials in commercial premises showed that no significant differences were found between traps with and without the lure either in terms of the number of insects caught or the number of positive traps. Representatives of the major storage beetle pest species were found at all sites and the effectiveness of the use of monitoring devices for early detection of insect presence was confirmed. However, further optimisation of the lure is required before a clear benefit for its inclusion can be shown on a consistent basis.

This study, although making significant progress towards a multi-species lure, has shown that this will not be a simple task. The electrophysiological study clearly demonstrated that not only were there differences in the compounds to which the insects responded between the three species, but also between the different sexes. This is not unexpected as the different species have different requirements for both food source and oviposition sites. Therefore the volatiles to which they respond must provide them with a clear signal that the appropriate conditions are present. The study has also shown that the peanut-carob mixture used as the starting material is not itself attractive to all species. There is therefore a requirement to identify other compounds for inclusion if all species are to be attracted and the effect of these on all target species would need to be examined.

One of the other considerations for further

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<tr>
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<td>Responded to carob-peanut extract</td>
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work is the ratio in which the compounds are mixed. In the study reported here the compounds were mixed in an approx. 1:1 ratio in the majority of the trials. It may be that, as has been suggested for the use of pheromones (Phillips et al., 2000), the components should be mixed in a ratio that more accurately represents the natural product. The necessity for this with food derived attractants remains to be determined.

Detection of mite infestations

Mite infestation is also a cause of concern to industry both for the damage caused and for the presence of allergens. Less research for monitoring of mites has been undertaken compared to that for insects. Sieving and flotation analysis can be used for the detection of mites in stored commodities (Lynch and Thind, 1984; Thind 2000; Anon 2003). Studies have shown that in stored grain and oilseed the PCT™ trap is effective in determining mite presence (Clarke, 2002; Dunn et al., 2005) and may provide an earlier indication of their presence than traditional methods (Dunn et al., 2005). The development of the BT trap (Thind, 2005) provided the first trap designed specifically for the detection of storage mite populations in storage and production premises. The trap has proven to be very effective in both laboratory and field studies (Thind and Ford, 2004; Wakefield and Dunn, 2005). However, the presence of mixed species populations resulted in under-representation of some species, in particular Lepidoglyphus destructor (Schrank), found in the trap (Wakefield and Dunn, 2005). The possibility of the addition of an attractant to the bait was therefore investigated. A review of the literature identified several potential compounds which could act as attractants for storage mites (Dunn, 2000). This review considered pheromone communication, for which there is still relatively little known for the Astigmata, in addition to the potential of food and fungal volatiles. This review has been followed by laboratory studies to ascertain whether compounds could be used as attractants for storage mites. The most promising compound identified, 2-nonanone, has been further studied by incorporation in BT traps and PCT™ traps. Laboratory studies examined the effect of the presence of these compounds on both single and mixed species populations and in the presence or absence of a food source (wheat or oilseed rape). In the absence of a food source, 2-nonanone incorporated in the BT trap improved catches of L. destructor and A. siro but had no effect on T. longior. In the presence of oilseed rape, trap catch for all three species was improved with the incorporation of 2-nonanone, when tested with individual or combined species. Inclusion of 2-nonanone in a PCT™ trap improved detection at the early stages of infestation in laboratory studies (Dunn, unpublished). Dunn et al. (2005) reported on studies comparing the effectiveness of the BT trap and the PCT™ trap in 20 tonne bins containing oilseed rape and compared traps with and without the addition of the 2-nonanone lure. The addition of the lure significantly improved trap catch of A. siro, T. longior and L. destructor in both BT traps and PCT™ traps. Effective methods of mite detection in grain bulks have therefore been identified, but as for insects interpretation of the numbers found remains to be established and the effectiveness of the attractants under practical conditions needs to be improved.

Biology and behaviour

In addition to optimising the sensitivity of traps it is important that they are optimally deployed, assessed and the findings are correctly interpreted. Successful deployment depends on our knowledge of insect behaviour. Spatial distribution of insects in both grain and in premises has been studied and recommendations for trap deployment have been made (Arbogast et al., 1998, 2000a,b, 2002a,b and 2004; Campbell and Hagstrum, 2002; Campbell et al., 2002; Nansen et al., 2003, 2004; Trematerra and Sciarretta, 2002, 2004; Trematerra et al., 2004; Athanassiou et al., 2005; Toews et al., 2005), but there are still areas of insect behaviour related to
their likelihood of being found in traps which are still poorly understood. We have recently examined several aspects of insect behaviour that could affect the likelihood of being caught in traps, for example, the response to temperature and moisture gradients and temperature thresholds for crawling locomotion and flight.

Response to temperature and moisture gradients

In the UK one of the primary control methods to reduce the risk of insect and mite infestation is to dry the grain to below 14.5% moisture content and to cool the grain below 15 °C within 2-3 weeks and to below 5 °C by end of December (Anon, 2003). During the drying and aeration process temperature and moisture gradients are created throughout the bulk. Even in the absence of physical methods such as cooling and drying, temperature and moisture gradients will be present as the surface layers will tend to follow ambient temperature and moisture uptake in the surface layers during the winter months will occur. There have been many studies on insect movement in grain bulks and to examine the response to temperature and moisture gradients for some species (for example Cox and Collins, 2002 and references therein; Arbogast, 2003 and references therein). However, there was a need to establish the behaviour of insects in grain bulks under current UK conditions and practices. We have examined the response of insects to temperature and moisture gradients and their movement in aerated and non-aerated bins in both laboratory and practical scale evaluations for O. surinamensis, S. granarius and C. ferrugineus (Conyers, unpublished data). Laboratory studies in the presence of grain examined both vertical and horizontal movement in response to temperature and moisture gradients. With no gradients of temperature or moisture it was found that S. granarius moved little from the introduction point in either vertical or horizontal arenas. Both O. surinamensis and C. ferrugineus tended to move more in the vertical orientation than the horizontal orientation. When a temperature gradient was added (15 °C in the cool zone up to 30 °C in the hot zone) S. granarius remained in the middle (temperate zone) with arenas in the vertical position but moved towards the cool zone in the horizontal position. Both C. ferrugineus and O. surinamensis moved towards the hot zone in both horizontal and vertical arenas but this effect was more apparent for C. ferrugineus. When a moisture gradient (40, 60 and 80 %) was used, with the temperature remaining at 20 °C, C. ferrugineus showed a preference for the zone with high relative humidity. S. granarius showed the highest movement away from the low relative humidity zone. O. surinamensis again showed no particular preference. Examples of insect distribution for the three species in horizontal arenas are shown in Figure 1. C. ferrugineus was most likely to move laterally or vertically through grain to locate the areas of preference. O. surinamensis was the most active of the three species tested and had the greatest tendency to move downwards through the grain. S. granarius was the least active species and it was concluded that they would be mainly found at or just below the surface of the grain. This agrees with observations from trapping studies in bulk grain where S. granarius tends to be found in the surface traps (Wakefield and Cogan, 1999).

To confirm the laboratory observations trials were undertaken in 25 tonne grain bins, 4 m deep and of section 3 x 3 m. Six bins were used two of which were unaerated and the remainder were aerated continuously at ca 10 m³/h/t. The two unaerated bins and two of the aerated bins contained wheat at 13% moisture content with an upper layer at 17% moisture content. The remaining two aerated bins contained grain at 13% moisture content only. Insects were introduced at the bottom of the bins and insect movement was assessed using insect traps positioned at the interface between the two grain layers and positions above this up to the surface. As had been indicated by the laboratory studies it was found that S. granarius did not move through the grain into the upper layer regardless of temperature, moisture or aeration status. Fewer O. surinamensis were usually trapped in all aerated bins than the unaerated bins. This could be explained by the lower temperatures found in the aerated bins. In unaerated bins most insects were generally caught at the centre of the bins but this did not apply to the aerated bins. A higher catch of O. surinamensis in the aerated
wet bins compared to the aerated dry bins was not explained by temperature differences and this was attributed to the higher moisture content. This data has enabled recommendations on trap placement to be made and will also assist in the development of models and decision support systems to examine the interactions of physical parameters and trap catch.

**Temperature thresholds for insect movement**

Recommendations for the frequency for checking traps can be related to ambient temperature. If the temperature is below that required for crawling locomotion or the flight threshold of insects then a decreased inspection frequency for traps can be recommended. We have recently established thresholds for crawling locomotion for seven species of storage beetle adult and four species of larvae (Table 2). These studies have shown the importance of monitoring in grain bulks and premises even at low ambient temperatures as insect movement may still occur. Insect movement in relation to temperature is one factor that will determine the number of insects caught. However, the relationship between trap catch and temperature is not fully established. Recommendations concerning trap interpretation and temperature must be used with caution until further information on insect behaviour at the trap is available.

Flight thresholds have also been established for the predominant beetle, moth and parasitoid species (Cox et al., in press; Table 3). In the UK

**Figure 1.** Distribution of *O. surinamensis*, *S. granarius* and *C. ferrugineus* in horizontal arenas filled with wheat with either a) no temperature or moisture gradients b) temperature gradient from 15 °C to 30 °C and c) moisture gradient from 40 to 80 % r.h. In all cases insects were introduced to the middle zone.
introduction of storage beetles to premises through flight is only likely for the foreign grain beetle, *Ahasverus advena*. However, it should be noted that these thresholds were established in the absence of food volatiles or semiochemicals and it is known that the presence of such volatiles can increase the propensity to fly (Cox and Dolder, 1995; Marsh et al., 1978).

**Can effective lures be designed?**

Although considerable progress has been made in the development of both pheromone and food-based lures the effectiveness of these still requires optimisation. Considerable promise has been demonstrated in laboratory studies but under practical based studies lures and attractants have not always proved as effective. This could be as a result of a number of factors, for example, environmental conditions, presence of food, the physiological state of the insects and strain differences and these will be examined in turn. Consideration should be given to these factors when designing laboratory and practical scale trials.

1. Environmental conditions. Fluctuating temperatures, available headspace and airflows will have an effect on volatile release rates. In the study to develop a multi-species lure we examined volatile release rates from the lures through headspace analysis using portable solid phase microextraction samplers. This showed that, as expected, the volatile release rate decreased with a decrease in temperature. However, the degree to which this affected the individual compounds was different (Wakefield et al., 2006) and therefore the volatile blend released differed with changes in temperature. This would certainly alter the effectiveness of the lure and laboratory studies should perhaps consider this in more detail during the optimisation process. Temperature will also affect the process by which insects respond to odorants and the effect of temperature on the component parts of this process has been reviewed (Cox et al., 2003). The available headspace for release of the volatiles will also tend to be less in the laboratory than in a practical situation. This will influence the overall effectiveness of the lure in addition to affecting field study parameters such as trap spacing. The presence of air currents will prevent build up of volatiles near the trap and although this may be effective in distributing the volatiles it is likely that the amounts present will be insufficient for the insects to respond.

2. Presence of food. The availability of food affects the behaviour of insects, pheromone

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<th>Table 2. Lowest temperature at which movement was recorded for various storage beetle adults and larvae over a 24 hour period.</th>
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<tr>
<td><strong>Species</strong></td>
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<tr>
<td><em>S. granarius</em></td>
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<tr>
<td><em>S. oryzae</em></td>
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<tr>
<td><em>R. dominica</em></td>
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<td><em>O. surinamensis</em></td>
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<td><em>O. surinamensis</em></td>
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<td><em>A. advena</em></td>
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<td><em>A. advena</em></td>
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<td><em>C. ferrugineus</em></td>
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<td><em>T. castaneum</em></td>
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<td><em>T. castaneum</em></td>
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production (for example Mayhew and Phillips, 1994) and the response to volatiles. In general, the majority of laboratory behavioural bioassays are done in the absence of a foodstuff and this may be the primary reason why attractants appear to be more effective in laboratory studies.

3. Physiological state of the insects. Insect age (for example, Boughton and Fadamiro, 1996; White, 1989; Wakefield et al., 2005b; Walgenbach and Burkholder, 1986), mated status (Walgenbach et al., 1983) and density (Pierce et al., 1983) have all been shown to affect the response of insects to volatiles. Laboratory bioassays tend to use insects of a known age range that have been reared at relatively high densities. The close proximity of the insects would ensure that nearly all are mated. In a practical situation insects will tend to be at lower densities and of a wide age range and this may affect the response of individuals to the lure.

4. Strain differences. In work using both pheromone and food attractants differences in response between strains have been observed (Boake and Wade, 1984; Wakefield et al., 2006). It may be possible that laboratory reared insects, which have been kept for many years under high-density conditions, would show a suppression in response, particularly to pheromones, through habituation (Rahalkar et al., 1985). Laboratory reared insects may also become less active than field strains and foraging behaviour may be reduced. For example, in experiments to examine insect movement in response to environmental gradients we have found that although there was no difference between field and laboratory strains of O. surinamensis in their response to temperature the field strain showed more movement towards high moisture and greater dispersion in the vertical plane, particularly downwards. A field strain of S. granarius showed a positive movement to cooler areas when responding to high temperatures compared to the laboratory strain and more dispersion in both the horizontal and vertical planes. Our studies to develop the multi-species lure included experiments in large grain bulks into which known numbers of insects were introduced. These insects were all laboratory strains that had been in culture for many years and which were the same as used in the laboratory studies. Future work should examine the use of realistic strains at the earliest possibility.

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<tr>
<th>Temperature (°C)</th>
<th>Moth</th>
<th>Parasitoid</th>
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<tr>
<td>12.5</td>
<td>E. kuehniella</td>
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<td>15.0</td>
<td>P. interpunctella</td>
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<td>E. elutella</td>
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<td>17.5</td>
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<td>A. calandrae</td>
<td>A. advena</td>
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<td>-</td>
<td>L. distinguendus</td>
<td>T. stercorea</td>
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<td>20.0</td>
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<td>-</td>
<td>R. dominica</td>
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<td></td>
<td>-</td>
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<td>C. ferrugineus2</td>
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<td>22.5</td>
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<tr>
<td>25.0</td>
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<td>T. castaneum</td>
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<tr>
<td>27.5</td>
<td>-</td>
<td>-</td>
<td>S. oryzae</td>
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1 Data from Cox et al, in press
2 Data for C. ferrugineus from Cox and Dolder (1995)
Interpretation of trap catch

The effectiveness of traps either with or without the addition of a lure is now well documented. However, research is still required to establish effective monitoring strategies that are based on insect behaviour and ecology (Campbell et al., 2002). The relationship between the number of insects found in the trap and the number present in the commodity or product has not been established making interpretation of trap catch difficult (Toews et al., 2005). By understanding the behaviour of insects and mites and through effective population modelling based on practical (fluctuating) environmental conditions it should be possible to interpret trap catch, relating it to populations present in the commodity or building and establishing control actions required. Establishment of spatial distribution, which will be identified through the use of monitoring programs, and the likely changes in this that may occur due to changes in environmental and physical conditions is one element that needs to be effectively established to enable a reliable risk assessment to be made. In addition reliable population models need to be determined. Population growth has generally been measured in laboratory studies with an optimum food source and constant temperature and moisture conditions. Data is required for population growth both in commodities and premises under variable environmental conditions and differing qualities and quantities of food. Only once the relationship between the number of insects found in traps and the number present in the product has been established can effective interpretation be possible leading to increased confidence by users for decision-making regarding prevention or control.

Future directions

Aggregation pheromones of the predominant storage beetle pest species have been identified but our knowledge is still expanding. Olsson et al. (2006) have recently suggested that male-produced volatiles from Tribolium confusum (du Val) may function as a sex pheromone rather than as an aggregation pheromone as reported for T. castaneum (Herbst). Bryning et al. (2005) have identified a sex pheromone from male Tenebrio molitor in addition to the previously reported female sex pheromone, 4-methyl-1-nonanol (Tanaka et al., 1986). Greater understanding of the pheromone biology of all species including the identification of minor components for some species (Chambers et al., 1996) will assist the development of lures and may provide insights for novel control mechanisms if for example methods to interfere with the behavioural responses can be identified. New synthetic routes for beetle pheromones may also further development of lures on a cost-effective basis. The response to different blends and ratios of both pheromones and food attractants also requires further research. The other area that requires research is the development of lure dispensers that can release volatiles consistently over a period of several weeks and over a broad temperature range.

In the future it may be possible that molecular techniques could provide a means to rapidly determine whether an insect will be able to perceive a chemical and whether there is likely to be a behavioural response. Over the past decade our understanding or the mechanism of insect olfaction has advanced considerably. Odorant binding proteins, which may transport odour molecules through the sensilla lymph, have been identified in several insect orders (Honson et al., 2005), and olfactory receptors (OR) have also been identified (Clyne et al., 1999). There is still much to learn regarding binding of odorant molecules and the signal transduction mechanisms and how these may differ in different insect species. However, this approach should further knowledge of insect olfaction allowing more targeted research and possibly offering potential to interfere with normal behavioural interactions.

Conclusion

Substantial progress has been made with regard to the detection of storage insects and mites using monitoring devices and a range of pheromone and
Acknowledgements

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