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Modelling Biological Control of Stable Flies by Means of Parasitoids

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Authors: Henrik Skovgård (Department of Agroecology, University of Aarhus)

Gösta-Nachman (Department of Biology, University of Copenhagen)

Photos: Henrik Skovgård

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Contents

Forord

Denne rapport beskriver og diskuterer resultaterne af projektet "*Biologisk bekæmpelse med parasitoider: Hvordan udnyttes de optimalt til bekæmpelse af stikfluer i malkekvægstalde?*".

Projektet er et samarbejde mellem Institut for Agroøkologi, Aarhus Universitet og Biologisk Institut, Københavns Universitet. Det er udført med støtte fra Miljøstyrelsen program for Bekæmpelsesmiddelforskning i perioden 2012-2014 (MST-667-00135).

Forfatterne takker Claus Dahl, Lars Damberg og Gitte Christiansen (alle institut for Agroøkologi) for deres store arbejde i laboratoriet. Vi takker desuden Anders Hansen, Marke, for at have stillet sin stald til rådighed for vores forsøg.

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Foreword

This report describes and discusses the results of the project: *Biological control using pupal parasitoids: How can they be used optimally to suppress stable flies in dairy cattle operations?*

The project is a result of collaboration between Department of AgroEcology, University of Aarhus and Department of Biology, University of Copenhagen. The project has been supported by the Danish Environmental Protection Agency as part of *The Danish Environmental Protection Agency Pesticide Research Program*. The project was conducted from 2012-2014 (MST-667-00135).

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The project has been attached to the advisory board "Agriculture" established in 2012 by the Agency. We thank all in the group for their good comments and suggestions during the project period.

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Dansk resumé

Stikfluen, *Stomoxys calcitrans* (L.), betragtes på verdensplan som et af de alvorligste skadedyr i forbindelse med kvægavl på grund af dens blodsugende adfærd. Fluernes smertefulde bid nedsætter dyrenes tilvækstrater og køers mælkeydelse. De økonomiske tab, som derved påføres kvægavlerne, er skønsmæssigt opgjort til over 2,4 milliarder dollars årligt alene i USA. Der foreligger ingen pålidelige opgørelser over de økonomiske tab i Danmark som følge af stikfluer, men årligt bruges der betydelige mængder af insekticider til at bekæmpe fluer i forbindelse med konventionelle kvæg- og svinebesætninger. Ud over stikfluernes økonomiske betydning kan deres bid og blotte tilstedeværelse være til stor gene for mennesker.

Problemer med fluer kan reduceres eller helt undgås ved at opretholde en høj sanitær standard i produktionsfaciliteterne. Det sker ved jævnligt at rense dem for husdyrgødning og efterfølgende opbevare den fjernede gødning under forhold, der forhindrer fluer i at udvikle sig i den. På nogle gårde kan det imidlertid være svært eller bekosteligt at forhindre fluer i at optræde i uacceptable mængder. Det gælder især produktionssystemer, hvor dyrene går frit i store åbne staldanlæg, og en egentlig fluebekæmpelse kan derfor komme på tale. På konventionelle (dvs. ikke-økologiske) gårde benyttes som regel kemiske bekæmpelsesmidler (insekticider), såsom larvicider mod flue-larver og adulticider mod de voksne fluer. Insekticider er nemme at benytte, men medfører en række utilsigtede virkninger, såsom sundhedsmæssige risici for mennesker, skader på miljøet og risikoen for at insekterne udvikler resistens mod bekæmpelsesmidlerne. Økologiske husdyrproducenter undgår disse negative effekter, men kan så til gengæld opleve meget store flueproblemer til gene for dem selv, deres familie og deres naboer, samtidig med at dyrenes velfærd nedsættes.

Et bæredygtigt alternativ til insekticiderne er at benytte fluernes naturlige fjender til biologisk bekæmpelse. I Danmark er der udført forsøg med at udsætte en lille snyltehveps (parasitoid) ved navn *Spalangia cameroni*, som lægger æg i flue-pupper, og resultaterne har hidtil vist sig lovende med hensyn til bekæmpelse af både stuefluer og stikfluer. For at opnå en tilstrækkelig effektiv bekæmpelse er det dog nødvendigt at benytte en såkaldt inundativ bekæmpelses-strategi bestående i, at store mængder af parasitoider udsættes gentagne gange. Typisk udsættes der 50-200 hunlige parasitoider pr. m^2 med gødning eller strøelse med to til fire ugers interval gennem flue-sæsonen, som strækker sig fra midt i april til midten af oktober. Det betyder, at der på den enkelte gård let kan blive tale om udsætning af over en million parasitoider om året.

Selv om *S. cameroni* er forholdsvis nem at masseproducere ved at bruge pupper af stuefluen eller gyllefluen som værtsorganisme, så er prisen pr. produceret hun stadig for høj til, at biologisk bekæmpelse kan betale sig rent økonomisk i forhold til kemisk bekæmpelse. Man må dog forvente at produktionsprisen falder i takt med at efterspørgslen stiger og mere effektive masseproduktionssystemer udvikles, således at biologisk bekæmpelse med tiden kan blive et konkurrencedygtigt alternativ til kemisk bekæmpelse, især hvis omkostningerne ved benytte insekticider øges, samtidig med at der lægges flere restriktioner på deres brug.

For at reducere omkostningerne ved at benytte biologisk bekæmpelse mest muligt, bør mængden af udsatte parasitoider afpasses efter behovet. Ideelt set bør bekæmpelsen sigte på at stikfluernes tæthed ikke overstiger en bestemt tærskelværdi, bestemt ud fra økonomiske og/eller genemæssige overvejelser. Imidlertid er sådan en skadetærskel, under danske forhold, endnu ikke bestemt for stikfluer. Så indtil videre må man nøjes med at bestemme sammenhængen mellem antallet af udsatte parasitoider og deres forventede effekt på bestanden af stikfluer for derved at bestemme den udsætningsstrategi, der giver mest mulig kontrol med færrest parasitoider.

Eftersom en stald er et komplekst økosystem, som påvirkes af mange faktorer, både biotiske og abiotiske, vil relationen mellem en bekæmpelsesindsats og den efterfølgende effekt på stikfluerne sædvanligvis påvirkes af en hel række andre faktorer, som ikke kan kontrolleres eksperimentelt. Det kan derfor være nødvendigt at udføre rigtig mange tidskrævende forsøg under naturlige forhold for at kunne finde frem til en metode, der optimerer brugen af parasitoider. Alternativet til den eksperimentelle fremgangsmåde er at udvikle en model af systemet, og så bruge modellen til at simulere systemet.

Baseret på eksisterende data hentet fra litteraturen og suppleret med data fra nye eksperimenter har vi udviklet en simuleringsmodel for systemet. Modellen, som hedder *The Fly Simulator*, beskriver dynamikken af stikfluer og *S. cameroni* under påvirkning af den omgivende temperatur. Modellen holder styr på antallet af individer i hvert udviklingsstadie (æg, larver, pupper og voksne) indenfor hver art og opdaterer udviklings-, overlevelses- og æglægningsrater på daglig basis afhængig af temperaturen i luften og i forskellige dybder i gødningen. Stikfluernes dødelighed afhænger af den intraspecifikke konkurrence mellem larverne om resurserne og deres risiko i puppestadiet for at blive angrebet af parasitoider. Angrebsraten af den enkelte hunlige parasitoid vokser med tætheden af egnede flue-pupper (det funktionelle respons) og aftager med tætheden af konkurrerende parasitoider, der søger efter værter indenfor det samme område (mutual interference). Modellen er interaktiv og giver mulighed for at brugeren kan benytte forskellige metoder til at bekæmpe stikfluerne, såsom at udsætte parasitoider, behandle gødningen med larvicid eller udmuge gødning. De tre metoder kan bruges hver for sig eller kombineres.

Modellen blev brugt til at analysere systemet under antagelse af, at de miljømæssige faktorer (temperatur og mængden af gødning) holdes konstant. Analyserne viser at systemet enten vil tendere mod et stabilt ligevægtspunkt, hvor populationerne forbliver konstante over tid, eller vil variere med konstant periode og amplitude (kaldet cyklisk stabilitet). Dynamikken afhænger af temperaturen. Analyserne viser også, at stikfluer kun kan opretholde en population, hvis temperaturen er mellem 13 og 34°C, men at temperaturer omkring 28-29°C giver dem optimale vilkår. *S. cameroni* kræver temperaturer mellem 22 og 32°C for at kunne sameksistere med stikfluer. Dens optimale temperatur ligger tæt op ad stikfluens.

Den simulerede dynamik af stikfluer blev sammenlignet med et empirisk datasæt, hvor antallet af stikfluer i en stald med 35-50 kalve på en økologisk gård er fulgt over en periode på to år. Eftersom modellen giver god overensstemmelse med observationerne, blev dette datasæt brugt som reference for at teste effekten af forskellige bekæmpelses-scenarier. Som grundlag for a sammenligne bekæmpelses-strategier brugte vi *FlueDage*, defineret som det daglige antal voksne stikfluer kumuleret over en given periode, som i dette tilfælde var to år. Bekæmpelsessuccessen blev målt ved hjælp af et kontrolindeks (*CI*), som udtrykker reduktionen i *FlueDage* som følge af bekæmpelsen divideret med den maksimale værdi af *FlueDage* (dvs. hvis der ingen bekæmpelse finder sted). *CI* udtrykkes i procent.

Vi brugte modellen til at undersøge om *S. cameroni* har mulighed for at etablere sig permanent i danske stalde og, hvis det ikke er muligt, til at undersøge om den kan anvendes i forbindelse med såkaldt inokulativ biologisk bekæmpelse (dvs. nogle få parasitoider udsættes tidligt i fluesæsonen, hvorefter de efterfølgende opformerer sig til så stort antal, at de kan bekæmpe skadevolderne effektivt). Simuleringerne viste, at parasitoiderne ikke er i stand til at overleve de lave staldtemperaturer om vinteren, mens den inokulative metode gav dårlige resultater, fordi parasitoiderne er ude af stand til at formere sig tilstrækkeligt hurtigt efter udsætningen.

To forskellige inundative udsætningsstrategier blev sammenlignet: fastlagte og adaptive udsætninger. Den førstnævnte strategi er baseret på, at tidspunkterne for udsætningerne fastlægges allerede ved sæsonens begyndelse, mens den sidstnævnte strategi bygger på, at udsætninger kun finder sted, når tætheden af fluer overskrider en bestemt tærskelværdi. Dog skal der være et minimum tidsinterval mellem to på hinanden følgende udsætninger for at give behandlingen tid til at virke inden næste udsætning. Ved begge strategier var antallet af parasitoider pr. udsætning fastsat på forhånd og derfor uafhængig af det aktuelle antal af fluer. På grundlag af de udførte simuleringer konkluderer vi, at den adaptive udsætningsstrategi kan spare nogle udsætninger, men gevinsten er lille i forhold til en fastlagt strategi. Så når man tager de praktiske og logistiske problemer i forbindelse med at anvende en adaptiv strategi i betragtning, er den fastlagte strategi at foretrække. Således vil en fastlagt strategi baseret på at udsætte ca. 200 parasitoider/m² (både hanner og hunner) hver fjerde uge medføre en forventet reduktion i antallet af stikfluer på 42%, mens den dobbelte udsætningsstørrelse ifølge modellen medfører en reduktion på ca. 70%. Disse udsætningsstørrelser ligger indenfor, hvad der er blevet benyttet i praksis. Til sammenligning opnår man en reduktion på ca. 97% ved at øge udsætningsstørrelsen til 750 parasitoider/m².

Vi undersøgte også, hvordan anvendelse af larvicid kan påvirke stikfluernes populationsdynamik. Det viser sig, at fluerne kan blive effektivt bekæmpet ved at benytte lave doser af larvicid anvendt med korte intervaller (fra 1 til 3 uger mellem hver behandling), mens større doser er nødvendige, hvis tiden mellem to behandlinger øges til 4 eller flere uger. Et interessant fænomen er, at høje insekticid-koncentrationer under visse omstændigheder kan give en dårligere bekæmpelse end hvis lavere koncentrationer var blevet benyttet.

Simuleringer, der undersøgte effekten af udmugning, viste at problemer med stikfluer kan reduceres eller helt undgås ved at fjerne en stor del af gødningen ved ofte og regelmæssige udmugninger.

Når en fastlagt udsætningsstrategi af parasitoider blev kombineret med kemisk bekæmpelse som del af en integreret bekæmpelses-strategi baseret på at larvicid kun anvendes, hvis antallet af stikfluer overskrider en bestemt tærskelværdi, viste den integrerede strategi sig at give bedre resultater end når den biologiske eller den kemiske bekæmpelse blev brugt hver for sig. Jo lavere tærskelværdi (f.eks. når antallet af stikfluer overskrider 100 eller 200 voksne fluer/m^2), jo oftere er man nødt til at supplere udsætninger af parasitoider med pesticidbehandling. Ved højere tærskelværdier (f.eks. 400 fluer/m^2) viste simulationerne, at pesticidbehandlinger helt kan undgås, når 300 voksne parasitoider/m^2 udsættes hver tredje uge.

Projektet har vist, at dynamikken af stikfluer med og uden bekæmpelse kan modelleres med rimelig realisme, selv om den nuværende version af *The Fly Simulator* skal betragtes som en prototype. Modellen vil kunne videreudvikles fra at være en strategisk model til en operationel (taktisk) model, som kan benyttes af den enkelte producent som en hjælp til at træffe de rigtige *ad hoc* beslutninger baseret på løbende informationer om systemet. Et sådan beslutningssystem, som med tiden kan udvides til at inkludere flere arter end kun stikfluer og *S. cameroni*, forudsætter først og fremmest at de øjeblikkelige tætheder af de involverede arter kan bestemmes både hurtigt og rimelig nøjagtigt, for derefter at benyttes i modellen sammen med pålidelige vejrudsigter til at forudsige, hvordan populationerne vil udvikle sig i den nærmeste fremtid med og uden indgreb.

Konklusioner og perspektivering

- Stikfluer forventes at blive et voksende problem i fremtiden, hvis kemisk bekæmpelse ved hjælp af insekticider ikke længere kan benyttes på grund af resistens, miljømæssige restriktioner eller forøget efterspørgsel blandt forbrugerne efter økologisk producerede mejeri og kød produkter.
- Alternativer til insekticiderne findes og omfatter ændringer i den måde, som gødningen behandles og opbevares (fysisk bekæmpelse) og biologisk bekæmpelse ved hjælp af fluernes naturlige fjender.
- Tidligere undersøgelser viser, at den mest oplagte kandidat til biologisk bekæmpelse af fluer i danske staldsystemer er parasitoiden *Spalangia cameroni.*
- En simuleringsmodel af samspillet mellem stikfluer og parasitoider, kaldet *The Fly Simulator*, giver en realistisk beskrivelse af systemets dynamik.
- Temperaturen er den vigtigste miljøfaktor, som påvirker fluernes populationsdynamik. Modellen forudsiger at stikfluer kan opretholde en levedygtig population i temperatur-intervallet fra 13 to 35^{o}C, mens den optimale temperatur ligger på ca. 28^{o}C.
- Når modellen køres med temperatur-data fra en stald på en dansk gård med økologisk kvægavl er den i stand til at forklare 64% af variationen i det observerede flue-antal (bestemt ved hjælp af fangst-genfangst).
- Når modellen benyttes til at simulere biologisk bekæmpelse ved hjælp af *S. cameroni*, viser det sig at parasitoiderne er ude af stand til at opretholde en permanent population i stalden. De må derfor genudsættes hvert år.
- Simuleringer viser, at *S. cameroni* kan bruges til biologisk bekæmpelse af stikfluer, hvis parasitoiderne udsættes gentagne gange og i stort antal igennem hele flue-sæsonen (fra midt i april til midt i oktober).
- Vi fandt at udsætning af 400 voksne parasitoider pr m^2 hver fjerde uge er tilstrækkeligt til at reducere forekomsten af fluer med omkring 70%. Dette resultat er i overensstemmelse med anbefalinger baseret på empiriske data.
- En lignende reduktion kan opnås ved hjælp af larvicide.
- Endnu bedre bekæmpelse af stikfluer kan opnås hvis kvægavleren sørger for at fjerne gødning med regelmæssige intervaller.
- Simuleringerne viser tydeligt at stikfluer ikke behøver at være et alvorligt problem i kvægproduktion, hvis udsætning af parasitoider kombineres med en rimelig høj sanitær standard i stalden.
- Vi fandt at en adaptiv strategi, baseret på at udsætte parasitoider hver gang tætheden af stikfluer overskrider en vis tærskelværdi, kun er marginalt bedre med hensyn til det totale antal udsatte parasitoider end en forud planlagt strategi, hvor et konstant antal parasitoider udsættes med faste tidsintervaller , uafhængigt af antallet af stikfluer. Vi foreslår derfor at benytte en fast planlagt strategi, fordi den kræver mindre logistisk planlægning, og fordi det ikke er nødvendigt at monitere antallet af stikfluer.
- Til trods for at planlagte udsætninger af parasitoider kan reducere tætheden af stikfluer betydeligt, forventer vi at de samme, eller endog bedre, resultater kan opnås til lavere omkostninger, hvis flue-bekæmpelse er baseret på en taktisk strategi.
- En taktisk bekæmpelses-strategi vil kræve at de øjeblikkelige bestande af skadedyr og nyttedyr kan estimeres ved hjælp af simple indsamlingsmetoder. En taktisk management model skal også kunne inkorporere vejrforudsigelser for tiden indtil næste populations-monitering finder sted. Baseret på modellens forudsigelser skal det afgøres om fluerne forventes at holde sig under en acceptabel tæthed. så det ikke er nødvendigt at bekæmpe dem eller, hvis det ikke er tilfældet, hvilke metoder der skal tages i anvendelse for at opnå den ønskede tæthed af fluer.

- Andre forudsætninger for at biologisk bekæmpelse kan lykkes er, (i) at effektive naturlige fjender kan masseproduceres til en rimelig pris, (ii) at de kan leveres i tilstrækkelige mængder, når der er behov for dem, og (iii) at de kan udsættes uden store omkostninger. Så længe disse forudsætninger ikke er opfyldte, kan det ikke forventes at kvægavlere vil skifte fra kemisk til biologisk bekæmpelse.
- Omkostningerne ved at bruge parasitoider til at bekæmpe stikfluer skønnes at være cirka dobbelt så høje som at benytte larvicider, men prisen for parasitoider forventes at falde i takt med at flere kvægavlere skifter til biologisk bekæmpelse, hvorved metoden med tiden bliver økonomisk konkurrencedygtig. I en overgangsperiode kan det være nødvendigt at pålægge kemisk bekæmpelse flere restriktioner, for eksempel ved at øge beskatningen af pesticider og bruge det ekstra provenu til at give økonomisk støtte til landmændene og/eller til producenterne af de naturlige fjender.
- Information om, hvordan man bedst kan anvende naturlige fjender til at bekæmpe fluer, er altafgørende for at sikre metodens succes blandt kvægproducenterne. Vi foreslår at landmændene tilbydes net-baserede kurser og online information om emnet, herunder at give dem mulighed for at "lege" med en spilversion af computer-programmet *The Fly Simulator*.

Summary

The stable fly, *Stomoxys calcitrans* (L.), is world-wide considered as one of the most serious pests associated with cattle production because of its blood-sucking behaviour. The flies' painful bites can reduce milk production in dairy cows, decrease weight gain in beef cattle and reduce feeding efficiency. The economic losses due to stable flies in the US alone are estimated to be more than $2.4 billion per year. In Denmark, there is no information about the costs caused by stable flies, but it is known that conventional cattle and pig farms use considerable amounts of insecticides to control flies. Apart from the economic losses caused by stable flies, the insects are considered as a major nuisance to humans.

Problems with flies can be minimized by maintaining a high sanitation standard in the production facilities, including frequent removal of manure and proper storage of the removed manure. However, on many farms, and in particular on farms where cattle are housed in free-stalling barn systems, removal of manure may occur too sporadic to prevent outbreaks of flies unless other control measures are implemented. On conventional farms, flies are usually controlled by frequent applications of insecticides which are used against either the larvae (larvicides) or the adults (adulticides). Insecticides are easy to use, but have many drawbacks, including health risks to humans, damage to the environment and the risk of insects developing resistance. Organic farms avoid these negative effects, but it may be at the expense of severe fly problems, thereby harming the farmers, their families and neighbours, and jeopardizing the animals' welfare.

A sustainable alternative to insecticides is to use biological control by means of the flies' natural enemies. In Denmark there is some experience in applying the pteromelid wasp *Spalangia cameroni* Perkins, which parasitize fly pupae. Some promising results have been obtained with respect to controlling stable flies and house flies. However, in order to keep the flies at acceptable levels, it was necessary to apply an inundative release strategy where large numbers of parasitoids were released at several occasions. Typically, at each release occasion about 50-200 female parasitoids were applied to each m^2 covered with manure (straw beddings). As the fly season usually extends from mid-April to mid-October and releases are recommended to take place at two to four weeks intervals, the total number of released parasitoids on a farm can easily exceed one million per year.

Though *S. cameroni* is easy to mass-produce, using house fly or black dump fly pupae for breeding them, the price per female is still too high to make biological control as cost-efficient as chemical control. However, if the demand for parasitoids increases and better mass-production systems are being developed, it seems likely that biological control over time will be economically competitive, especially if the costs of purchasing insecticides increase or some restrictions are put on their use.

In order to reduce the costs of applying biological control, the number of released parasitoids should be adjusted to the needs. Thus, if damage or economic thresholds for the abundance of stable flies can be established, biological control can be optimized so that the number of released parasitoids does not exceed what is necessary to keep the flies below the chosen threshold. At the moment, the data to estimate such thresholds are not available, so instead we investigated the relationship between the number of released parasitoids and the expected effect on the fly population in order to identify the release strategy that gives most control by means of fewest parasitoids.

As the stable system is very complex and influenced by many factors, both biotic and abiotic, the relationships between control measures and the observed density of stable flies are usually obscured by the concurrent influence of many confounding factors which cannot be controlled experimentally. Therefore, an experimental approach to solve the problem of optimizing pest control requires many replicated experiments, making such an approach very costly in terms of time or money. An alternative to the empirical approach is to develop a model of the system and then apply the model to simulate the system.

Based on existing data obtained from the literature and supplemented with data derived from new experiments, we developed a simulation model of the system. The model, called *The Fly Simulator*, describes the dynamics of stable flies and *S. cameroni*, and uses the ambient temperature as the driving variable. The model keeps track of the number of individuals in each developmental stage (eggs, larvae, pupae and adults) of both species and updates the rates of

development, survival and oviposition at a daily basis dependent on the daily temperatures in the air and at different depths in the manure. Mortality of stable flies depends on the intraspecific competition among larvae for resources, and on the risk of being attacked by a parasitoid. The attack rate of an adult female parasitoid increases with the density of suitable host pupae (i.e. the functional response) and declines with the density of parasitoids due to mutual interference among the competing females searching in the same area. The model is interactive and has options allowing the user to introduce parasitoids, treat the manure with larvicide or remove manure during a simulation. The three control options can be applied either singly or in combination.

The model was used to analyse the system assuming that environmental conditions (temperature and amount of manure) do not vary over time. The analyses show that the system can either approach stable equilibria, where the populations remain constant over time, or exhibit stable limits cycles, where the populations will fluctuate with constant period and amplitude. The type of dynamics is strongly influenced by the temperature. The analyses further show that stable flies require temperatures between 13°C and 34°C to maintain a permanent population, although temperatures around 28-29°C are most favourable. *S. cameroni* requires temperatures between 22°C and 32°C to coexist with stable flies. It temperature optimum is close to that of stable flies.

The simulated dynamics of stable flies was compared with a data set obtained over two years in a stable with 35-50 calves at an organic farm. As the model gave good fit to data, we applied this data set as a reference for testing various control scenarios. As a means for comparing control strategies, we used *FlyDays*, defined as the cumulated number of adult flies occurring every day over a given period of time (two years). As a measure of how much the various control efforts reduced fly abundance we calculated the control index (*CI*) as the reduction in *FlyDays* divided by the maximum *FlyDays* (i.e. in absence of control). *CI* is expressed in percentage.

We used the model to investigate whether *S. cameroni* is likely to establish permanently in Danish stables or whether stable flies can be controlled by means of inoculative biological control (i.e. relatively few parasitoids are released early in the fly season, whereupon efficient control is achieved during the remaining season without additional releases). The first approach did not succeed, because the parasitoids were unable to survive the low stable temperatures experienced during winter time, while the inoculative approach failed because the parasitoids after introduction were unable to achieve densities that were sufficient to exert effective control of the flies.

Two different inundative release strategies were compared: Pre-planned and adaptive releases. The former strategy is based on a fixed schedule for when releases take place, while the releases in the latter strategy take place only when the density of flies exceeds a certain threshold. Releases should, however, be separated by a minimum number of days between two successive releases to allow a treatment time to work. In both strategies, the number of parasitoids used per release was chosen in advance and was therefore independent of fly density. On basis of the simulations we conclude that an adaptive strategy is slightly better than a pre-planned one. However, if the practical and logistic problems of applying an adaptive strategy are taken into consideration, we suggest that a pre-planned strategy should be preferred. Thus, a pre-planned strategy based on releasing ca 200 parasitoids/m^2 (both sexes) every 4 weeks is expected to reduce fly numbers with ca 42%, while a doubling of the release size can lead to ca 70% reduction. These release sizes lie within the range of what has been used in practice. In comparison, a reduction of ca 97% can be achieved by increasing the release size to 750 parasitoids/m^2.

We also investigated how use of a larvicide will interfere with the population dynamics of stable flies. We found that effective control can be achieved by applying low doses of larvicide when applied at short intervals (once every one to three weeks), whereas higher doses will be needed if the time between successive treatments is four or more weeks. Interestingly, the simulations indicate that high doses of larvicide may sometimes result in poorer control compared with lower doses.

Simulations taking removal of manure into consideration confirm that maintaining a high sanitation standard in a stable by frequent cleanings can reduce or even prevent problems with stable flies.

When a pre-planned release strategy was combined with chemical control as part of an integrated pest management (IPM) strategy, based on the condition that larvicide should only be used when the number of flies exceeds a certain threshold, we found that integrated control usually gave better results in comparison to biological or chemical control used as the only strategy. The lower the intervention threshold (when the abundance of stable flies exceeds e.g. 100 or 200 adult flies/m^2), the more often will pesticide applications be needed. At higher treatment thresholds (e.g. 400 flies/

m^2), the simulations showed that pesticide applications can be completely avoided in an IPM strategy based on a release size of 300 adult parasitoids per m^2 every third week.

The project has demonstrated that the dynamics of the stable fly system with and without control interventions can be modelled with reasonable realism, though the current version of *The Fly Simulator* has to be considered as a prototype. Thus, we end up by giving a number of suggestions as how to proceed from the current strategic model toward an operational (tactical) model which can be used by the individual farmer to assist him in making the right ad-hoc decisions based on available information. Such a support system, which may be extended to include more species occurring in livestock facilities than just stable flies and *S. cameroni*, will first of all require that the current population densities of the species in focus can be monitored in a fast and reliable way and then entered into the model to be combined with weather forecasts, costs of buying and applying natural enemies and pesticides, as well as farm-specific information to yield reliable predictions of what will happen with and without interference. Although there is still a long way to go before this goal can be accomplished, a step has hopefully been taken by the model presented in this report.

Conclusions and perspectives

- Stable flies are likely to become an increasing problem if chemical control by means of insecticides can no longer be used due to development of resistance, environmental contamination or an increased demand among consumers for organically produced diary and meat products.
- Alternatives to insecticides are available and include changes in the way manure is treated and stored (physical control) and biological control by means of natural enemies.
- The most obvious candidate for biological control in Danish cattle facilities is the parasitoid *Spalangia cameroni*.
- A simulation model of the interactions between stable flies and parasitoids, called *The Fly Simulator*, provides a realistic description of the system's dynamics.
- Ambient temperature is the main driving variable for fly dynamics. The model predicts that stable flies can maintain a viable population in the temperature range between 13 and 35°C, but the optimal temperature is around 28°C.
- When the model is run with temperature data obtained from a Danish organic cattle farm, the model is able to fit the observed number of flies (estimated by means of mark-recapture) quite well, explaining 64% of the variation in data.
- Using the model to simulate biological control by means of *S. cameroni* revealed that the parasitoids will be unable to maintain a permanent population in the stable. They therefore have to be reintroduced every year.
- The simulations demonstrate that *S. cameroni* can be used for biological control of stable flies if the parasitoids are released in large numbers at several occasions (inundative releases) throughout the fly season (from mid-April through mid-October).
- We found that releases of 400 adult parasitoids per m^2 every four week are sufficient to reduce fly abundance with about 70%. This result is accordance with recommendations based on empirical data.
- Similar reductions in fly abundance can be achieved by means of a larvicide.
- Even better control of stable flies can be achieved if the farmer takes care in removing manure at frequent intervals.
- The simulations clearly demonstrate that stable flies do not need to be a serious problem in cattle production systems if parasitoid releases are combined with a reasonable high sanitation standard.
- An adaptive release strategy based on releasing parasitoids only when the abundance of stable flies exceeds a certain density was found to be marginally better (in terms of the total number of released parasitoids) than a pre-planned release strategy where a fixed number of parasitoids are released with fixed intervals, irrespective of the abundance of stable flies. However, we recommend a pre-planned release strategy, because it seems easier to apply from a logistic point of view and because it does not require monitoring of stable flies.
- Although pre-planned releases of parasitoids can reduce the abundance of stable flies significantly, we expect that the same, or even better results, can be achieved at lower costs if fly control is based on a tactical approach.
- A tactical approach will require that reliable estimates of the current population sizes of pests and beneficial organisms can be obtained by means of a simple sampling method. A tactical management model should also incorporate weather forecasts for the period until the next monitoring takes place. Based on the model's predictions, decisions can be taken as to whether flies are expected to remain below acceptable limits, so that no control measures are needed or, alternatively, what kind of control measures should be implemented to achieve this goal.
- Additional conditions for applying biological control are (i) that effective natural enemies can be mass-produced at a favourable price, (ii) can be delivered in sufficient numbers when they are needed, and (iii) finally can be released with little effort. It cannot be expected that the majority of conventional farmers will switch from chemical to biological control as long as these conditions are not met.

- The costs of applying parasitoids to control stable flies are estimated to be approximately twice the costs of applying larvicides, but the price of parasitoids is likely to decline as more farmers switch to biological control, making this method more economically competitive. However, in a transition period, it may be necessary to put more regulations on the use of pesticides, e.g., by increasing the taxes on pesticides and use the extra revenue to give economic support to the farmers and/or the producers of natural enemies.
- Information about how to manage fly populations by means of natural enemies is paramount to ensure the success of the method. We suggest that the farmers are offered web-based training courses and on-line information, including the possibility of playing with a game version of *The Fly Simulator*.

1. Introduction

1.1. Background

High-density confinement systems for livestock production are today a common picture throughout most of the world, including Denmark. These systems provide high cost-efficiency in animal production, but at the same time they increase the problems of manure handling and disposal, as well as causing problems with flies. High concentrations of animal waste increase the abundance of nuisance flies, affecting the livestock, the farm operators and the neighbours living close to farms (Axtell 1986). Fly species closely associated with confined livestock are termed synanthropic flies or just nuisance flies, which basically include two fly species in Denmark: the house fly *Musca domestica* L. and the blood feeding stable fly *Stomoxys calcitrans* (L.). This project focuses on the stable fly because of its key role as a serious nuisance pest in Danish cattle production and because it is considered as an economically important species world-wide.

Control of flies around confined-animal operations is not a simple issue. Basically, it is a management problem which further is complicated by different types of animal production facilities and husbandry practices, as well as by regional and sometimes climatic differences (Axtell 1986, Wilhoit et al. 1991). Nevertheless, the principles of fly control are universal and if properly understood can be adapted to specific circumstances. Successful fly control requires a management approach normally involving an integration of different control methods in a manner compatible with the current animal production practices (Axtell 1986, Wilhoit et al. 1991).

Stable fly management in livestock production in the US is a major issue of the agricultural enterprise and estimated to cost the cattle industry more than a billion dollars per year (reviewed in Taylor et al 2012). It makes stable flies as the most important damaging pest associated with cattle production (Lysyk and Schaalje 1992, Mullens and Peterson, 2005).

In Denmark, the agricultural practice has been through substantial changes during the last decades where relatively small farms with mixed husbandry production have been replaced by large operations housing several hundred animals. Thus, modern Danish dairy cattle facilities have on average more than 150 cows – but several farms have already 300-400 heads. A number that is likely to be even higher in the future. The majority of the modern cattle farms have their animals in free-stall systems with limited or no access to pasture (Dansk Kvæg 2004; Dansk landbrug i Tal, 2009).

To achieve a high milk production per lactating cow, animals are given surplus of supplementary food as silage, which is normally based on fermented grass or corn plants. This may incur a high spillage of fodder which, mixed with animal urine, manure and straw, becomes excellent development sites for stable flies (Thomsen 1938, Axtell 1986). The same applies to free-stalls with resting areas or calf pens having deep beddings (a mixture of fermenting straw, urine and manure) (H. Skovgård pers. obs.).

Among the factors prompting the development of an integrated pest management approach (IPM) to control stable flies in confined animal production systems are: (*i*) limited choice of effective insecticides and application methods, (*ii*) rapid development of resistance to insecticides, (*iii*) the need to avoid insecticide residues in animal products, (*iv*) the modern production systems facilitate high densities of stable flies, (*v*) greater awareness of environmental impacts of pesticide uses, (*vi*) concern for cost-effectiveness of pest control in livestock production, and (*vii*) a growing awareness that stable flies cannot be eradicated, but need to suppressed to an acceptable level (Axtell 1986).

The prime objective of IPM is to reduce the overall abundance of stable flies to an acceptable level defined by either their nuisance effect (damage threshold) or their economic impact (economic threshold) with a minimum input of insecticides. Choices of appropriate control measures have to be made by taking the biology of the pests, the complex of natural enemies, competing species and the production system into consideration.

In animal production systems with prolonged accumulation of manure, it is normal to find a diverse fauna of arthropods. Among these are several species of mites and rove beetles preying on eggs and larvae of flies. In addition, several species of parasitoids lay eggs on fly pupae, causing the eventual death of their host (Axtell & Rutz 1986, Geden & Axtell 1988). Besides, various diseases take a large toll of flies, primarily among the young stages but also the adults (Skovgård & Steenberg 2001).

The parasitoid species associated with livestock facilities in Denmark are typically small (2-3 mm) hymenopteran wasps belonging to the family Pteromalidae. One of these species is *Spalangia cameroni* Perkins, which is common in northern Europe where it attacks pupae of many different fly species including the house fly and the stable fly (see section 2.2.1). Recent field studies conducted in Denmark and Norway have demonstrated that *S. cameroni* has the potential for controlling both fly species. The parasitoids were released in large numbers and repeatedly in order to achieve sufficient control (Skovgård & Jespersen, 2000, Skovgård, 2004, Skovgård & Nachman, 2004, Birkemoe et al. 2009).

To optimize the use of *S. cameroni* for biological control of stable flies a more thorough understanding of the interactions between the two species, and the influence of the environmental conditions on these interactions, will be paramount. Especially the timing of releases and the number of parasitoids to be released are important. Conducting field trials for identification of an optimal release strategy are costly both in terms of time and money, and the results are often associated with high uncertainty due to lack of replications. To circumvent these short-comings, a simulation model can be applied (e.g. Lysyk & Axtell 1987, Wilhoit et al. 1991).

The main advantage of applying a model is that it can be used to run scenarios within few seconds that otherwise would take months to study experimentally. Besides, the state of the simulated system is explicitly defined so as to exclude all factors that otherwise will obscure experimental results. The model's assumptions, as well as its parameters, can easily be changed, thereby allowing the user to explore the consequences in terms of predicted dynamics and to test the robustness of the model's assumptions. Simulation models are excellent tools for testing control strategies and at the same time to give basic insights into the dynamics of the modelled system, thereby identifying the most important biological processes involved. Finally, once a model has been verified and validated it may be used as a predictive tool to forecast the expected state of the system from its current state with a time-horizon determined by the precision of the model and the influence of stochastic phenomena (e.g. the effect of weather on indoor conditions).

1.2. Project goals

The objectives of the project are:

1. **To develop a simulation model of the interactions between stable flies, *S. cameroni* and the influence of the environment on the dynamics of the system.**
2. **To parameterize the model, applying data from existing literature and supplemented with data from our own experiments.**
3. **To use the model to investigate the underlying dynamics of stable flies and *S. cameroni* in a constant environment.**
4. **To apply the model to simulate how populations of stable flies will develop under environmental conditions prevailing in a dairy cattle operation and to compare the predicted dynamics with empirical data.**
5. **To incorporate *S. cameroni* in the model to simulate its dynamics and its effect on the abundance of stable flies.**
6. **To investigate whether *S. cameroni* can establish a persistent relationship with its host or whether it has to be reintroduced into the system every year.**
7. **To use the model to test the effect of various release strategies on the abundance of stable flies.**
8. **To optimize the release strategy of *S. cameroni* with respect to release sizes and timing of releases.**
9. **To investigate how biological control by means of *S. cameroni* can be used in combination with other control options, such as insecticides and removal of manure.**
10. **To use the model to give recommendations as to how *S. cameroni* can be used as part of an integrated control strategy (IPM).**

As a spin-off of the above objectives, we transformed the simulation model to a user-friendly program called the *The Fly Simulator*. The program, which can be implemented on an ordinary PC, is interactive so that the user can play with the model and imagine he or she is a farmer that has to make some decisions as to how stable fly populations should be managed.

The project is primarily based on available literature, but also on new experimental data obtained within the frame of the present project. Thus, three papers are direct outcomes of the project: the first dealing with the functional response of *S. cameroni* (Skovgård & Nachman 2015a), the second dealing with mutual interference among female *S. cameroni* (Skovgård & Nachman, 2015b), and the third dealing with development, survival and oviposition of *S. cameroni* (Skovgård & Nachman submitted to Environmental Entomology). During development of the model it was realized that important information about the system is either non-existing or inadequate to make the model realistic in all aspects. Therefore, the model has to be seen as the state-of-the-art, but with room for improvements as more or better data become available.

1.3. Structure of the report

The reader is introduced to the stable system and the modelled species in Chapter 2. Chapter 3 gives a verbal description of the conceptual model. The model is validated against empirical data in Chapter 4. In Chapter 5, the model's internal behaviour is studied in details to examine its dynamics when exposed to different constant temperatures, while the model's sensitivity to changes in its parameter values is explored in Chapter 6. In Chapter 7 different control strategies are compared. In Chapter 8, we discuss to what extent the model is realistic and fulfils it purposes. The report is rounded up by two separate chapters: Conclusions in Chapter 9 and Perspectives in Chapter 10. Appendix 1 describes the mathematical structure and functional relationships of the model in detail, while Appendix 2 is dedicated to explain how the model's parameters were estimated. Appendix 3 is a user-manual to *The Fly Simulator*.

2. The stable system

2.1 *Stomoxys calcitrans*

2.1.1. Taxonomy

Stomoxys is a genus of flies within the family Muscidae, a family that includes the very common and well-known species *Musca domestica* L. or the house fly. The genus *Stomoxys* includes species that are bloodsucking ectoparasites on mammals. One of these species is *Stomoxys calcitrans* (L.) or the stable fly. This is the only species within the genus found in the northern and central Europe (Frantisek et al. 2002). It most likely originates from Africa, but is now cosmopolitan due to human movements and activities (Zumpt 1973).

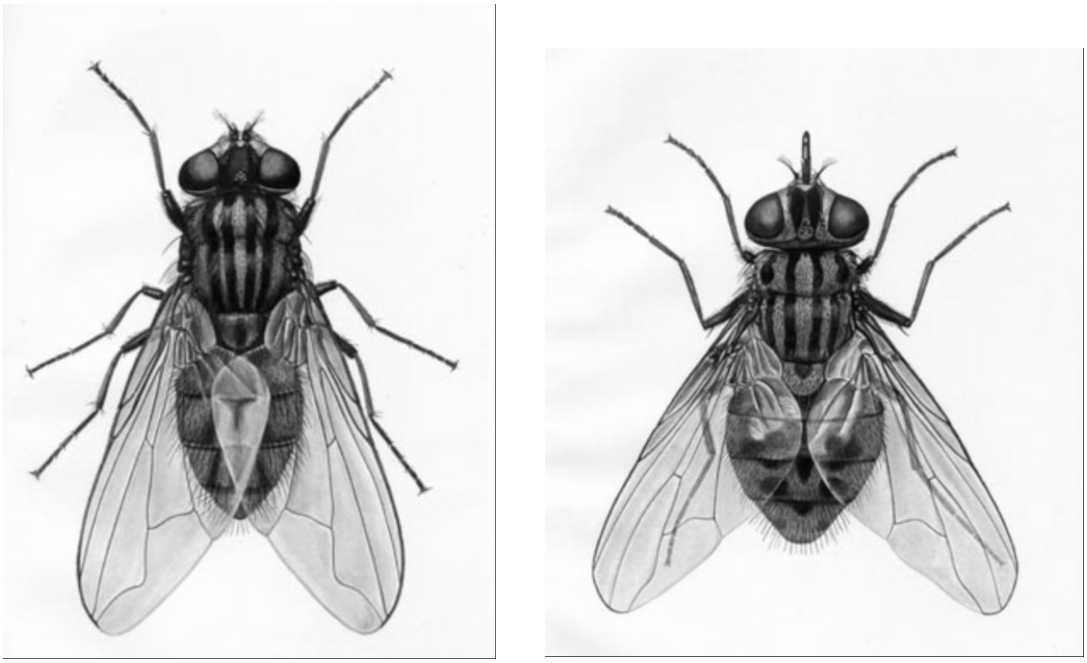

FIG. 2.1. THE HOUSE FLY (*MUSCA DOMESTICA* L.) (LEFT) AND THE STABLE FLY (*STOMOXYS CALCITRANS* (L.)) (RIGHT). FROM THOMSEN (1938).

For an untrained eye the stable fly looks like the house fly but the two species can easily be distinguished by their mouthparts (Fig. 2.1). The stable fly has a distinctive non-retractile black proboscis which it uses to pierce the skin of hosts to ingest blood. During resting, most of the proboscis is hidden below the head and thorax so only a minor part of it can be seen as a small tip projecting in front of the head. Stable flies can further be distinguished from house flies by their checkerboard pattern of eight dark spots on their dorsal parts of abdomen. Both genders of the stable fly are obligate blood feeders and feed during day time only (Thomsen 1938).

2.1.2. Life-cycle and development

The stable fly has complete metamorphosis, i.e., the life cycle includes the following stages: egg, larva, pupa and adult. The egg is white, elliptical and about 1 mm long. It hatches within 1-2days depending on the temperature. Because the larvae are sapro- and coprophages, favorable habitats for oviposition are silage, bedding mixed with urine and faeces, rotting hay, fermenting feed or other decomposing and fermenting plant materials (Thomsen 1938, Axtell 1986). At high population densities, females are observed to lay eggs in all kinds of media leading to low survival of the emerged first stage larvae (H. Skovgård, pers. obs.). The larval stage consists of a first-, second-, and third-instar, which are white and have a cylindrical body shape. Lower and upper temperature threshold for larval development is 10°C and 30°C, respectively (Thomsen 1938, Gilles & Duvallet 2005a). At optimum temperature, larval development normally takes 6-8 days. Prior to pupation, the larva begins to move out of the organic media in search for dryer and cooler (15-20°C) areas.

During pupation the larval integument (the larval skin) contracts to form a cylindrical puparium which is about 3-5 mm long. The puparium gradually darkens to a color of light brown to dark brown. One to two days after the formation of the puparium, a pupa will develop inside. Full development of the fly pupa typically requires 4-21 days, depending on temperature, whereupon the newly developed adult pushes off the anterior end of the puparium. This is done by means of the ptelinum, an inflated sac that protrudes from the frontal region of the head due to blood pressure and muscle contractions. After the fly has left the puparium, it seeks hide to unfold its wings which takes approximately a day. The overall life cycle of the stable fly takes about 13-18 days during summer in areas where the mean air temperature is 24-30°C, but may take 20-25 days in Denmark due to the lower summer temperatures (Thomsen 1938, Kunz et al. 1977, Axtell 1986, Gilles & Duvallet 2005a). Development during winter time is very slow and hinges mainly on the larvae (Thomsen 1938, Berry et al. 1978), though it should be noticed that larval development is likely to be faster than predicted from the ambient air temperatures due to the heat produced by decaying and fermenting organic matter.

The females copulate once during life-time, usually 1-2 days after emergence. Egg production requires a blood-meal and typically a female starts egg-laying 4-8 days after copulation. After each blood meal a female will be able to produce an egg batch containing 25-50 eggs, all laid within the same day. A female typically deposits 4-6 batches of eggs during lifetime resulting in 100-300 eggs (Friesen & Johnson 2012). However, the total number of eggs per female depends on many factors, such as the temperature, the experimental set-up, the geographic area from which the flies originate, and the degree of inbreeding in the experimental flies (see e.g. Lysyk 1998, Gilles et al. 2005a). In the laboratory, females are reported to survive 20-30 d (Lysyk 1998), but under field conditions life expectancy ranges between 4 and 11 days (Berry et al. 1981, Scholl 1984, Skovgård & Nachman 2012). The mean sex ratio (males:females) is close to 1:1 (Lysyk 1998, Salem et al 2012).

2.1.3. Ecology

The number of stable flies is determined by abiotic factors like temperature, moisture and humidity (Wilhoit et al. 1991, Lysyk 1993, Berkebile et al. 1994, Lysyk 1998, Broce et al. 2005, Mullens & Peterson 2005, Taylor et al. 2007, Skovgård & Nachman 2012, Jacquiet et al. 2014), and by biotic factors which include pathogens, predators, parasitoids, food resources, and competitors (either conspecifics or alien species) (Thomsen 1938, Axtell 1986). Under natural conditions, fly populations are likely to be strongly influenced by natural enemies, but in animal production facilities the environment is altered, creating an unnatural situation favoring severe outbreaks of flies. The overall temperature range and prevailing summer temperatures in a given area are crude indicators of how fast fly populations will develop and how many generations per year they will produce (Axtell 1986).

The seasonal dynamics of stable flies under Danish conditions typically follows a unimodal pattern characterized by a few flies in late March and early April. These flies originate primarily from larvae that have survived the winter inside the stable or in nearby deposits of manure. From May and onwards a more or less exponential population growth takes place with a marked peak population in July or beginning of August followed by a gradual decline towards October and November (Fig 4.2). This pattern is in accordance with the general picture reported from temperate regions (Somme 1959, Titchener et al. 1981, Greene 1989). However, in the mid-western United States, where herds are confined in outdoor feedlots, fly abundances show a bimodal or even tri-modal pattern with peaks coinciding with rainy periods during the otherwise hot and dry summer (Rasmussen & Campbell 1981, Scholl 1986, Taylor et al. 2007). It indicates that rain along with temperature is a driving force for fly development, because precipitation prevents the food substrate from drying out.

In contrast to the dry regions of North America, northern Europe usually experiences precipitation throughout the year, so it seems likely that summer temperatures, rather than precipitation, triggers the development of stable fly populations (Lysyk 1993, Skovgård & Nachman 2012). In spring and early summer, the flies primarily develop at indoor sites where the animals provide ample supply of breeding material and ensure relatively high temperatures (Thomsen 1938). Later during summer, high outdoor temperatures may enable the flies to develop both inside and outside the stables, causing high population densities in mainly July and August (Skovgård & Nachman 2004). In this period, the milking herd is mainly on pasture from early morning to late afternoon, whereas heifers, calves, and nursing cows often remain indoors.

The decline in adult stable flies in September is caused by a combination of declining ambient temperatures, diseases, and natural enemies, e.g., pupal parasitoids (Skovgård & Jespersen 2000, Skovgård & Nachman 2004). From October-November until March, stable flies remain at very low levels, but if they are found at all it will usually be near the stocked

animals where the temperature stays above 6°C. Despite the fact that the air temperature in most animal facilities is below the threshold value (10°C) for fly activity and development (Lysyk 1993), it is likely that heat produced by the animals, in combination with various fermenting material, may enable a few flies to feed, mate, and lay eggs during the winter period (Scholl et al. 1981, Meyer & Petersen 1983, Berkebile et al. 1994).

In an earlier Danish study it was indicated that stable flies require temperatures between 10.2°C and 33.4°C to increase in population size and with a maximum per capita growth rate (r_m) of 0.070 d^{-1} at 21.8°C, and with a doubling time of 10 d (Skovgård & Nachman 2004). Based on laboratory experiments, Lysyk (1998) found a maximum r_m-value for *S. calcitrans* of *ca* 0.22 d^{-1} but occurring at 27.8°C. A likely explanation for the marked differences between the two studies could be that the latter is based on laboratory experiments while former was obtained under natural conditions, where the stable flies were exposed to competitors (e.g. house flies); natural enemies (spiders, pupal parasitoids, and birds); and diseases (entomopathogenic fungi) (see, e.g., Skovgård & Steenberg 2002, Skovgård & Nachman 2004).

2.1.4. Sampling and monitoring populations of stable flies

As part of any control program aiming at reducing the abundance of stable flies, it is necessary to monitor the pest population at regular intervals. Systematic monitoring can tell whether a chosen control strategy has been successful and may serve as an early warning so that control measures can be initiated before it is too late. Monitoring provides the information needed to time releases of beneficial organisms or applying a pesticide. Although visual observations made under non-standardized conditions can give an indication of whether flies are rare or abundant, such observations may often be inaccurate and thereby give a biased picture of the true situation. Therefore, in IPM programs systematic monitoring is an important component. Several methods for assessing the abundance of stable flies have been suggested: resting counts, sticky fly ribbons and spot cards, leg counts on animals, and mark-recapture (Axtell 1986).

Resting counts can be used in pre-designated areas of partitions. For instance, portions of feed troughs, railings, ceilings can be chosen and the stable flies resting in such areas are counted. Likewise, the flies resting on the animals or certain parts of an animal can be counted. If flies in many sampling areas or on many animals are counted, fairly reliable data can be obtained. However, the observer has to be present, counts are only for a brief period of time and the stable flies cannot always be accurately distinguished from other fly species. For indoor monitoring of stable flies and house flies, Kristiansen & Skovmand (1985) applied a logarithmic scale to grade fly abundance. Based on visual inspections of animals, the number of flies on a given animal is scored on a scale from 0 through 7, where 0: 0-3; 1: 4-6; 2: 7-12; 3: 13-25; 4: 26-50; 5: 51-100; 6: 101-200 and 7: 201-400 flies. Usually, up to 10 randomly chosen animals are included in a sample. The overall abundance of flies can either be expressed as the average of the individual scores, but for many purposes it may be better to convert the indices to the number of flies per animal by using the mid-points of the various intervals and then calculate the arithmetic average of these values (see Skovgård & Jespersen 2000).

Sticky ribbon cards can be purchased commercially and the flies caught on them can be used as an index of fly abundance. An advantage of this method is that the flies stuck on the cards in most cases can be identified. Several cards have to be placed in a facility and it is important that they are placed in areas of fly activity. The cards should not be left in a facility for more than 2-3 d, because they risk to be covered with dust or dead flies and thereby becoming inefficient. For outdoor monitoring of stable flies in USA and North-America, glued panel screens (sticky traps) emitting UV-light from the sun have been used with success (Williams 1973, Kaufman et al. 2005).

White spot cards measure fly activity. The cards are placed on sites in the stable where flies normally spend time resting after host feeding. Stable flies resting on spot cards will normally leave reddish fecal spots, which can easily be counted when a card has been exposed to flies for 3-5 days.

Leg counts are mainly used in USA where binomial sampling plans of stable fly counts on front legs or all legs of the same animals are used as a measure of activity and to calculate population levels on a relative scale. The method may also be used to estimate the economic threshold level (Lysyk & Schaalje 1992).

Mark-recapture methods, like Bailey's triple catch (Bailey 1952) or Jolly-Seber's stochastic method (Seber 1973), are considered as the most reliable way of estimating population sizes of flies (Skovgård & Nachman 2012, Nachman & Skovgård 2012). The methods are based on capturing flies by, e.g., sweep netting, mark them with a fluorescent powder, and then release them. After a while, usually one day, a new sample of flies is taken, and the proportion of marked and unmarked flies is used to estimate the total population size, as well as the dilution rate (births+immigrations) and the loss

rate (deaths+emigrations). Mark-recapture methods not only provide point estimates of the population size but also the standard error associated with such estimates. However, mark-recapture methods are tedious, laborious and costly to apply, so their main justification is when they can be used to determine a calibration line between a simple index value (e.g. resting counts, sticky ribbon or spot card samples) and the concurrent estimate of absolute population size. A complication associated with converting index values to absolute population estimates is that the former are based on fly activity, which is likely to be affected by ambient temperatures.

2.1.5. Economic importance

As previously mentioned the stable fly is an obligate ectoparasite where both genders ingest blood regularly from warm-blooded animals (Thomsen 1938, Meyer & Petersen 1983, Skovgård & Nachman, 2004). Studies indicate that even at relatively low densities of stable flies, the growth potential of cattle can be affected negatively (Campbell et al. 1977, Wieman et al. 1992, Catangui *et al.* 1997). According to Campbell et al. (1977) and Rutz & Geden (2000), one to three stable flies per leg are sufficient to cause economic loss in beef feedlot cattle. However, other authors state that more flies are needed to cause an economic damage (Catangui et al. 1997, Campbell et al. 2001). Some studies have shown that stable flies in dairy cattle productions can cause 6%-10% reductions in the milk production (Freeborn *et al.* 1928, Bruce and Decker 1958). In contrast, Mullens et al. (2006) did not find any reduction in milk production even though the number of biting flies was 2-4 per foreleg.

There is no information about the economic impact of stable flies in Denmark, but it is well known that many dairy cattle producers experience recurrent problems with stable flies during the summer and autumn period (July-September) where the density of stable flies can attain high numbers in the animal operations (Skovgård 2004, Skovgård & Nachman 2004). Furthermore, in modern animal systems with milking robots the presence of stable flies can cause the animals to kick off the machinery. In best cases, this will abrupt the milking process, but in worst cases damage the expensive equipment (H. Skovgård, pers com.).

Stable flies are known to be carriers of several bacteria that directly or indirectly can cause diseases in animals or humans (Hald et al. 2008, Förster et al. 2009).

2.1.6. Control methods

Cultural control of stable flies and other filth flies is based on manipulating the key abiotic factors like temperature and moisture in order to suppress fly abundance. Surely, without suitable substrate for fly development, there will be no flies, so efforts should be directed towards proper management of the manure by removing it frequently and thoroughly. An alternative is to change the conditions in the manure so it becomes unattractive to flies. Thus, ventilation or airflow in animal housing facilities may reduce air humidity and thereby dry out the substrate (Axtell 1986). Furthermore, protection of silage and hay balls, two potential stable fly development habitats, against rain may have a similar effect. When old manure is piled and compacted, usually outdoor, it will have a detrimental effect on fly development, because fermentation will increase temperatures beyond what can be tolerated by immature flies (Thomsen 1938). The temperature can be further increased by covering the piled manure with canvas. At the same time, covering will prevent flies access to the manure and prevent hatched flies from escaping the pile. Though covering is strongly recommended as a cost-effective method to reduce fly abundance, it is rarely adopted by farm operators.

Reliance on insecticides as the only option for controlling stable flies rarely leads to satisfactory results. Modern free-stall systems are large, high roofed facilities, which make it difficult to apply insecticides in a proper, efficient and environmental safe way. In conventional Danish dairy cattle facilities, the insecticides used are larvicides (growth regulators affecting molting of larvae by inhibiting proper development of a larva's exoskeleton), and attractants baited with adulticides to suppress populations of adult flies. If the timing and correct doses are used, larvicides seem to be a reliable but probably also the most expensive choice for a long term control of flies. However, the general impression is that many farmers do not possess the full knowledge about the biology of flies, and stable flies in particular, to apply insecticides optimally (H. Skovgård, pers. obs.). Furthermore, improper use of insecticides may lead to unnecessary contamination of the environment and may increase the risk of pesticide resistance to evolve (Keiding 1999).

Resistance to the insecticides has been reported for most fly species, including the stable fly, which is a fact of great concern. Although the synthetic pyrethroids were introduced as promising replacements for the environmentally

damaging organophosphates and chlorinated hydrocarbons, resistance against pyrethroids has developed and is today common (Cilek & Green 1994, Pitzer et al. 2010). Although new types of insecticides are being marketed (e.g. the neonicotinoides) it is probably only a question of time before control of stable flies, as well as of many other pest species, by means of insecticides will no longer be an option.

Information on the amount of biocides or active ingredients used for control of flies in Danish livestock production is not directly available. However, based on Miljøstyrelsens database on biocides approximately 8.7 tons active ingredients (pyrethrins, azamethiphos, thiamethoxam, imidachloprid, triflumeron and cyromazin) were used against flies and other arthropod pest species in 2010 (Miljøstyrelsen 2011).

Biological control is in most cases an environmentally sound and effective method of reducing pests and their unwanted effects through the use of other living organisms, which typically can be predators, parasitoids, fungi, and pathogen microorganisms (DeBach 1964). In many cases, biological control also involves an active human management role and may therefore be an important component of IPM programs as well. Biological control relies on three basic types of control strategies: importation or classical biological control, augmentation and conservation (DeBach & Rosen 1991). Classical biological control involves the introduction of a pest's natural enemy or enemies to a new area where they do not occur naturally. This strategy is especially interesting if the pest species is accidentally introduced into a new geographic area without the occurrence of its indigenous natural enemies.

In the 1960'ies and 70'ies, scientists imported and released several new species or strains of pupal parasitoids in North America in an attempt to achieve control of house flies and stable flies. The parasitoids were obtained from parasitized pupae of house flies and stable flies collected in, e.g., Australia, Asia or Europe (Legner 1978). To control flies in Denmark, Mourier & ben Hannine (1971) released parasitoid species new for the country, but the introductions failed and in most cases the released species could not be recovered after some time. The reasons could be that too few individuals were introduced, that the species were poorly adapted to the new environmental conditions or that the natural enemies were poorly synchronized with the occurrence of the pest species.

The strategy behind augmentative biological control is to boost the naturally occurring populations of enemies by supplementary releases. Augmentative releases can be of two types: Inoculative and inundative releases. The former is based on releasing relatively few natural enemies once or twice at a critical time during the season based on the expectation that the natural enemy will persist and multiply, resulting in a significant suppression of the pest population. In contrast, inundative release strategies often require releases of many thousands or sometimes even millions of natural enemies to avoid that the pest population exceeds the damage threshold (i.e. the density above which the pest causes unacceptable damage or becomes an intolerable nuisance) or the economical threshold (i.e. the pest density at which the economic revenues of controlling the pest balance the costs of controlling it). Inundative control methods require that natural enemies can be mass-reared in the laboratory at low costs, transported to the farms when needed, and be easily released (see Section 2.2.5).

Because the stable fly is an opportunistic species it has a relatively high population growth rate (see above) rendering an inoculative method with small chances of success. However, the inoculative use of black dump flies *Hydrotaea aenescens* Widemann has shown to be an effective method against house flies in slurry canals on pig and poultry farms (Nolan & Kissam 1985). Third larval stage of *H. aenescens* becomes highly predacious and attacks all stages of the house fly leading to almost eradication of the pest. A single release of *H. aenescens* in late spring on pig farms is normally sufficient to control the house fly throughout its entire activity period. Unfortunately, *H. aenescens* will not establish in dryer areas containing, e.g., fermenting organic matter, and can therefore not be used against stable flies (Skovgård pers. obs.).

Most attempts to control stable flies apply the inundative method which often involves massive releases of pupal parasitoids on a biweekly or monthly scheme. In this context, species from the genus *Spalangia* sp. have shown the most promising results against both stable flies and house flies (Morgan 1981). Thus, *Spalangia cameroni* has been mass-released on several Danish livestock facilities with either pigs or dairy cattle (Skovgård 2004, Skovgård & Nachman 2004). The general picture is that biweekly releases of 50-200 adult female *S. cameroni* per m^2 will be sufficient to suppress house flies to acceptable densities when the releases are initiated in early April before the first major outbreak of flies. With respect to the stable fly, releases of *S. cameroni* reduced the populations but not to a similar satisfactory level as found for the house fly (see also Birkemoe et al. 2009).

Conservation pest management can be subdivided into conservation and enhancement strategies. Conservation means that populations of natural enemies are protected by reducing the use of insecticides or by applying insecticides in a way

that will reduce their negative effects on the enemies. Enhancement means that the density of the natural enemies is enhanced by providing alternative food, refuges etc. (DeBach & Rosen 1991). Insecticidal control of adult flies by means of synthetic pyrethroids or control of larvae by means of larvicides may have detrimental effects on many natural enemies including parasitoids (Floate & Fox 1999. Therefore, in order to conserve the fauna of natural enemies, insecticides directed towards adult insects should be applied in bait stations attracting the adult flies or on treated surfaces. However, such a system has not yet been developed for controlling stable flies. For enhancement of natural enemies, it has been tested whether a manure removal schedule that leaves behind some of the old manure, allowing the natural enemies to reinvade the newly produced manure, will work, but the results so far have not been promising (Mullens et al. 1996).

2.2. *Spalangia cameroni*

2.2.1 Taxonomy

The genus *Spalangia* Latrielle, which is cosmopolitan, belongs to the family Pteromalidae. Many species are parasitoids on synanthropic filth flies and other flies (Bouček 1963). Several species have been considered for biological control of house flies and stable flies (Morgan et al. 1981, Andress & Campbell 1994, Mckay & Galloway 1999, Skovgård & Nachman 2004, Kaufman et al. 2012).

Individuals of *Spalangia* can easily be distinguished from parasitoids that belong to other genera occurring in the habitats occupied by flies. All members of the genera have an elongated, flattened body. The color of the body is always blackish and without a metallic green luster as seen in other genera of the pteromalids (Bouček 1963, Rueda & Axtell 1985). A unique feature of *Spalangia* spp. is that the bases of the antennae are placed near the mandibles whereas other genera have the antennae placed higher on the head. Most *Spalangia* species have golf-ball-like punctures scattered all over the head and on the thorax from which single seta points out (Fig 2.2).

FIG. 2.2. THE PARASITOID *SPALANGIA CAMERONI* PARASITIZING A PUPA INSIDE A PUPARIUM OF A HOUSE FLY.

Spalangia cameroni Perkins is secondary cosmopolitan, having been fortuitously or deliberately introduced into many parts of the world. It attacks various fly species belonging to the families Muscidae (including the house fly and the stable fly), Calliphoridae (blow flies) and Sarcophagidae (flesh flies) (Bouček 1963). *Spalangia cameroni* is one out of four native species of *Spalangia* recorded in Denmark. The three other species are *S. nigripes* Curtis, *S. nigra* Latrielle and *S. subpunctata* Förster, but these species are rare compared with *S. cameroni*, which occurs both widespread and abundantly (Skovgård & Jespersen 1999, Skovgård & Jespersen 2000).

2.2.2. Life-cycle and development

Spalangia cameroni is an idiobiont ectoparasitoid, which means that the female parasitoid uses its ovipositor to sting and paralyse its host (a fly pupa), preventing it from further development while the parasitoid larvae develops outside the fly pupa protected by the puparium (see Section 2.1.2). *Spalangia cameroni* has complete metamorphosis, i.e., the life cycle includes the egg, larval, pupal and adult stage. It is a solitary parasitoid which means it lays only a single egg on each pupa. The deposited egg hatches after about two days. The young larva searches actively for a suitable site on the host pupa, where it uses its mouthparts to pierce the pupal wall to start feeding. At 30°C, the time of development from egg and to adult emergence is 21-25 days, whereas it may take several months at 15°C (Geden 1997, Birkemoe et al. 2011). When development is completed, the adult parasitoid bites a circular hole in the puparium to exit. Males usually emerge a few days before the females. Mating takes place shortly after emergence.

Unmated females only produce male offspring, while a mated female can adjust the sex ratio of its offspring by laying female (diploid) eggs mainly in large hosts and male (haploid) eggs in smaller ones (King 1994). At emergence, a female *S. cameroni* usually have some eggs already ready for egg-laying (Gerling & Legner 1968).

Laboratory studies show that *S. cameroni* can stay alive for several months, but the average life expectancy under field conditions is probably no more than two to three weeks (Bouček 1963). A female can increase her survival time and life-time fecundity by feeding on host pupae (host feeding). She uses the ovipositor to penetrate the pupal wall and then licks up the haemolymph, which flows out through a sucking tube to the surface of the puparium (Gerling & Legner 1968).

Female *S. cameroni* lay 50-100 eggs in her life-time (Bouček 1963). Although the females possess the ability to discriminate between healthy and parasitized pupae, more than one egg may occasionally be deposited in the same pupa (superparasitism). Superparasitim occurs mainly when there is strong competition among females for suitable hosts. As a result of superparasitism, only one (or sometimes none) of the larvae will survive due to cannibalism (Gerling & Legner 1968, Petersen et al. 1991, Böckmann et al. 2012). The maximum daily production of offspring has been estimated to be 7-14 at 29-32°C (Moon et al. 1982, Geden 1996, Skovgård & Nachman 2015a). Most eggs will be laid during the first two weeks of egg-laying (H. Skovgård pers. obs). Very few offspring will be produced at temperatures below 15°C and above 35°C (Geden 1997, Birkemoe et al. 2011). The sex ratio is female biased with 60- 70% females (Legner 1967, Mann et al 1990, Skovgård & Nachman 2015a).

2.2.3. Ecology

Adult *S. cameroni* have well-developed wings, allowing them to spread effectively. They are attracted towards light (positive phototropic) (Bouček 1963). The parasitoids are mainly found inside animal facilities, where they prefer to search for hosts in loose substrates such as bedding materials (Smith & Rutz 1991). However, Mullens et al. (1986) observed that *S. cameroni* were actively parasitizing fly pupae mainly during the day, though this was questioned by Smith & Rutz (1991) who found no difference between day and night hours in parasitation activity. Many species of pupal parasitoids confine their activity to the upper 0-3 cm of the manure, whereas *S. cameroni* is capable of moving as deep as 10-15 cm (Skovgård 2006).

Seasonal activity of *S. cameroni* in Danish animal facilities begins in April, which coincides with the increase in outdoor temperatures and the occurrence of house and stable flies (Skovgård & Jespersen 1999, Skovgård & Jespersen 2000). From May and onwards, parasitism increases gradually and attains a maximum in September-October. This maximum is usually delayed by a month or so in relation to peak abundance of the two fly species (Skovgård 2004). From October-November the activity of *S. cameroni* levels off and becomes insignificant in December. During winter time no activity is observed unless the temperature inside the stable is high enough to sustain a population of flies (Skovgård & Jespersen 2000). Winter mortality of parasitized pupae was found to be almost 100% (Floate & Skovgård 2004).

The properties that make *S. cameroni* a promising candidate for biological control of stable flies in Denmark are: (*i*) it is a native species, (*ii*) it is a natural enemy of stable flies, (*iii*) it is mainly active indoors, (*iv*) it is willing to move into manure, (*v*) it can be fairly easily mass-produced.

2.2.4. Monitoring parasitoid activity

In order to assess the activity of pupal parasitoids under field conditions, a common method is to collect fly pupae from the manure and bring them to the laboratory for determination of the number of pupae that either hatch to flies, to parasitoids or do not hatched (Legner et al. 1967, Skovgård & Jespersen 1999). The unhatched pupae can be dissected to determine whether a parasitoid has been present. However, this method tends to overestimate the true activity of the parasitoids because the pupae may have been exposed to parasitism for a very long time, especially when ambient temperatures are low. To circumvent this problem, parasitoid activity can be monitored by the sentinel bag technique of Rutz & Axtell (1980) where small mesh bags containing laboratory reared fly pupae (usually 30) of known age (2-3 days old) are placed in the animal environment. The mesh bags allow the tiny parasitoids to move freely in and out, but prevent flies from escaping. The mesh bags can be placed where parasitoid activity is expected. After a week or shorter the bags are collected and the pupae are treated in the same way as described above. The main advantage of the mesh bag method is that it gives a standardized measure of activity during a known period.

2.2.5. Application and mass production

Three parasitoid species are currently marketed in Denmark as agents against house and stable flies. The species are *Muscidifurax raptorellus* Kogan & Legner, *Nasonia vitripennis* Ashmead and *S. cameroni*. Typically, the farmer signs a contract that offers him support and delivery of parasitoids during the fly season. On a biweekly schedule he will receive bags containing parasitoids, which have to be released at sites considered as hot spots for development of flies.

The recommended release size of *S. cameroni* is 50-200 female parasitoids per m^2 treated area and releases should preferably take place in late afternoon to avoid strong day light (Skovgård 2004, Skovgård & Nachman 2004). In North America, the quantity of released parasitoids is related to the number of animals on a farm (Cranshaw et al 1995). Both release criteria are operational from the perspective of the farmer and simple rules of thump, but do not reflect the actual need of parasitoids which depends on the current abundance of flies. This will require that flies are monitored at regular intervals (see Section 2.1.4) as strongly recommended by Axtell (1986).

The strategy of releasing three different parasitoid species at the same time seems logic as *M. raptorellus* and *N. vitripennies* prefer to stay on top of the manure, while *S. cameroni* is more willing to move deeper (Rueda & Axtell 1985, King 1997, Geden 2002, Skovgård 2006). However, in practice it seems difficult for the companies to deliver all three species simultaneously as test samples show that *N. vitripennis* is by far the most abundant species in the deliveries (H. Skovgård pers. obs.). The reason is most likely due to contamination because production of black dump fly pupae, *H. aenescens*, interferes with production of parasitoids. When the fly pupae are used for mass-producing the parasitoids, many of the pupae are already attacked by *N. vitripennis* or *M. raptorellus*. Unfortunately, both *N. vitripennis and M. raptorellus* seem to be the least efficient of the three parasitoid species (Mckay & Galloway 1999, Floate et al. 2000, Pitzer et al. 2011).

3. The model

3.1. Introduction

A simulation model consists of five main components: (1) The *state variables* describing the state of a system at a given instant of time; (2) The *rate variables* determining how the state variables change during a time step; (3) The *functional relationships* are the equations used to model how the changes in the system components are expected to occur; (4) The *parameters* are the constants of these equations; (5) The *driving variables* are state variables that affect the system, but are not affected by the system, e.g. time and ambient temperature.

In order to develop a model, it is necessary to define what the purpose of the model is so as to avoid that the model becomes unnecessary complex. Complexity increases with the number of state variables we want to include in the model. We have decided to limit the number of state variables to those describing the population sizes of only two species occurring in a stable: the stable flies (*S. calcitrans*) and its natural enemy, the parasitoid *S. cameroni*. Besides, our model should include information about the number of individuals in each developmental stage and of individuals in each age class within a stage. As each age class is characterized by the state of the individuals belonging to it, it is quite obvious that the model contains a large number of state variables.

As changes in state variables are brought about by the rate variables, which are linked to the state variables via the functional relationships and their parameters, it becomes even more obvious that presenting the model in details will be quite comprehensive. It is therefore moved to Appendix 1, which gives the mathematical description of the model's functional relationships, whereas Appendix 2 presents the parameter values and their estimation based on empirical data. Appendix 3 gives a user-friendly introduction to how the simulation model can be applied. Thus, Chapter 3 will focus on giving a verbal and visual presentation of the model, so as to guide "equation-phobic" readers through the model in a (hopefully) gentle way.

3.2. The stable environment

The model predicts the dynamics of populations of stable flies and parasitoids in a dairy cattle operation. The environmental factors are determined by temperatures in the air and in the manure. Air temperatures can be either constant or vary over time based on actual temperature records. The manure is produced by cattle confined in one or more areas (called compartments) inside the stable. The amount of manure (depth of bedding) can be either constant or vary over time. Temperature in the manure depends on the air and floor temperatures, the depth of the manure or bedding, as well as on the heat production of the fermenting medium and the rate at which temperature gradients are levelled out by heat conduction.

3.3. Population structures

A population of stable flies and parasitoids consists of males and females belonging to various developmental stages (see Section 2.1.2 and 2.2.2). The model differentiates between the four life stages (eggs, larvae, pupae and adults) of the stable fly, but since the parasitoid spends the immature stages inside a fly puparium and we have no information about the duration of each separate stage, we have for simplicity lumped the egg, larval and pupal stage of the parasitoid into a single stage called the "immature stage".

3.4. Temperature effects on population processes

For each time step of one day, the populations are updated to account for the aging of individuals. The model operates with two different age schedules: the *chronological age*, which is the number of days an individual has spent in a given life stage, and the *physiological age*, which depends on the ambient temperature experienced by the individual (van

Straalen 1983, Régnière & Logan 2003). The time an insect spends in a given stage typically decreases with temperature as long as the temperature is within a lower and upper threshold. Physiological age is measured in *degree-days* (Gilbert & Gutierrez 1973, Mironidis & Savopoulou-Soultani 2008) which is the cumulated daily average temperature above the lower temperature threshold. No development takes place when the temperature is below the lower temperature threshold, while death occurs when the temperature exceeds the upper threshold.

For each day an individual spends in a given life stage, it accumulates temperature in terms of degree-days. When it has accumulated sufficient degree-days it may hatch to the next stage. The model assumes that hatching occurs with a certain probability that increases with the physiological age of an immature individual. Adults do not hatch, but instead their mortality rate usually increases with their physiological age. The model accounts for *developmental variability* (Wagner et al. 1984, Schaalje & van der Vaart 1989, Son & Lewis 2005), because individuals entering a given stage at the same time will not leave it again (either by hatching or dying) at the same time (Fig. 3.1).

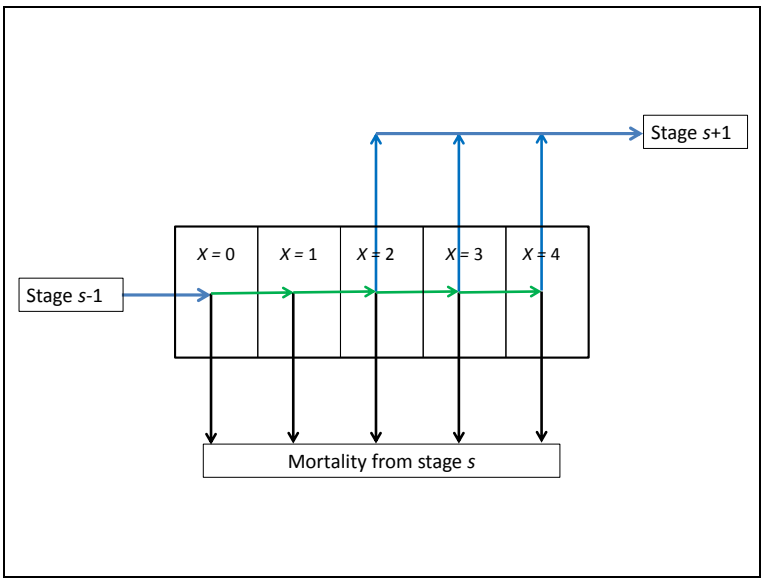

FIG. 3.1. SIMPLIFIED DIAGRAM SHOWING THE MODELLING OF LIFE STAGE *S* IN A POPULATION. INDIVIDUALS ENTERING THE STAGE FROM THE PREVIOUS STAGE DURING ONE DAY CONSTITUTE A COHORT AND ARE GIVEN AGE $X = 0$. FOR EACH DAY INDIVIDUALS SURVIVE, THEIR CHRONOLOGICAL AGE INCREASES WITH ONE DAY. AT THE SAME TIME, THEIR PHYSIOLOGICAL AGE INCREASES DEPENDING ON THE AMBIENT TEMPERATURE. INDIVIDUALS IN AN AGE CLASS WILL EITHER SURVIVE TO THE NEXT AGE CLASS (GREEN ARROWS), DIE (BLACK ARROWS) OR HATCH TO THE NEXT STAGE (BLUE ARROWS). THE LIKELIHOOD OF HATCHING INCREASES WITH THE PHYSIOLOGICAL AGE OF INDIVIDUALS. THE NUMBER OF AGE CLASSES DEPENDS ON THE TIME IT TAKES UNTIL ALL INDIVIDUALS HAVE EITHER DIED OR HATCHED. FOR FEMALE ADULTS, THE BLUE ARROWS INDICATE THE NUMBER OF EGGS PRODUCED PER DAY. THESE EGGS WILL ENTER STAGE 1 (THE EGG STAGE). ADULT MALES ONLY DIE.

3.5. Factors affecting survival

The model assumes that the mortality rate of immature individuals is independent of their physiological age, but ambient temperature affects their likelihood of surviving from one day to the next. For each life stage there is a temperature that is optimal, i.e. where the survival rate is highest. The more the temperature deviates from the optimal, the lower the survival rate. The same applies to the adult stage, so for individuals in this stage mortality depends on both the current temperature and on their physiological age.

Survival of stable fly larvae also depends on crowding effects (see Section 3.9). Furthermore, fly pupae attacked by a parasitoid are doomed to die (see Section 3.7). Insecticides applied to the manure decrease survival rate of fly larvae (see Section 3.11). Finally, removal of manure kills immature stages of both species and the adult *S. cameroni* (see Section 3.12).

3.6. Factors affecting fecundity

Ambient temperature and physiological age affect the fecundity of the adult females. Daily oviposition rates typically peak at an optimal temperature, while egg-laying stops when the temperature either becomes too low or too high. Physiological age first promotes fecundity, which typically increases steeply with age to reach a maximum at a young age and then gradually declines with age until the female eventually dies.

3.7. Effect of host density on parasitism

Adult female parasitoids need to oviposit in host pupae in order to reproduce. Thus, the fecundity of female parasitoids depends on the availability of suitable fly pupae and the time it takes to find and attack hosts. The attack rate of the female parasitoids increases with the density of the host pupae and will gradually approach a maximum rate when the host density becomes very high. The relationship between host density and attack rate is a type II *functional response* curve (Holling 1959), which is typical for most arthropod predators and parasitoids (Hassell 1978). The upper asymptote of the functional response curve depends on the ambient temperature and the physiological age of the females. Attacked pupae either produce a parasitoid offspring or die due to e.g. host feeding or because the parasitoid egg inside a puparium does not survive (see Section 2.2.2). Percent parasitism is calculated as the number of immature parasitoids divided by the total number of pupae (parasitized and unparasitized).

3.8. Effect of parasitoid density on parasitism

Parasitoid females that search for hosts within the same area may affect each other. This phenomenon, known as *mutual interference* (Hassell 1978), reduces the attack efficiency of the individual female and is modelled as a function of parasitoid density.

3.9. Effect of stable fly density on intraspecific competition

Food for stable flies is considered as unlimited except for the larval stage. Thus, when the density of larvae increases it will increase the *intraspecific* competition between the larvae, which will increase the per capita mortality rate due to a combination of starvation, interference and cannibalism (Mueller & Joshi 2000). Such a *density-dependent* factor prevents the fly population from reaching unrealistically high densities in absence of other mortality factors, e.g., natural enemies.

3.10. Manure temperatures

The immature stages of both species inhabit the manure, and their developmental and survival rates are strongly affected by the temperature in the manure. In general, the temperature here exceeds that of the surrounding air due to heat produced by fermentation of the organic material. We used a simple model that divides the manure into layers of 1 cm thickness. The temperature in a given layer is affected by the fermentation process and by the temperature exchange with the neighbouring layers due to heat conduction. The temperature in the uppermost layer depends on the air temperature in the stable, while the temperature in the lowest layer depends on the floor temperature. The model predicts that the temperature in the upper layers tracks the air temperature but show less temporal variation and a time delay compared with the air temperature, whereas the temperature in the deeper layers is usually higher and shows less temporal variation than in the top layers.

3.11. Effect of pesticide application on survival

The main purpose of the model is to provide insight into the host-parasitoid interaction. However, for demonstrating the interplay between biological and chemical control we have prematurely incorporated pesticide application into the model. It is assumed that the pesticide is a larvicide which affects the likelihood that larvae do not moult but die instead. As stable fly larvae have three instars before reaching the pupal stage, we model larvicide-induced mortality by increasing the mortality rate during the three moults taking place during development from egg to pupae, assuming that each larval stage has the same duration (in degree-days). Pesticide-induced mortality depends on the efficiency (lethality) of the applied larvicide and its concentration in the manure. The concentration depends on the applied dose, the amount of manure, and the rate at which the pesticide decays in the manure. Pesticide concentration may also decline due to removal of manure.

3.12. Effect of manure removal

Like pesticide application, removal of manure is not a key issue of the model. Nevertheless, we have included it as an option to illustrate how removal of manure may interfere with other control options. We assumed that all immature stages occurring in the removed manure will be killed. The same applies to the adult parasitoids, which are assumed to spend most time inside the manure, whereas the adult stable flies survive manuring by escaping. Compartments within the stable may not be cleaned at the same day and cleaning may not be complete, saving a small part of the immature stages in the remaining manure after a cleaning operation.

3.13. Spatial effects and dispersal

If the cattle operation consists of two or more compartments, the dynamics of populations in each compartment is modelled separately. The compartments share the same air temperature but may differ with respect to manure depth and thereby also with respect to manure temperatures. Only the adults of both species can spread from a compartment to another. This means that a cleaned compartment will quickly be recolonized by immigrants from other compartment(s). The model also allows for immigration of flies from outside.

Manure is a very complex environment to model as its quality for the insects inhabiting it depends on manure temperature, texture, water content, concentrations of oxygen and toxic substances such as methane and ammonia, etc. We therefore assumed that female stable flies lay eggs on or just beneath the surface of the manure where they are exposed to the average temperature of the upper 3 cm. Fly larvae are assumed to occupy the manure down to 8 cm and are exposed to the average temperature in the layer between 0 and 8 cm. The larvae move to the upper 3 cm of the manure to pupate, where they are exposed to the same temperature as the fly eggs. Adult stable flies occupy areas within the stable where they can optimize their fitness, defined as the temperature where the product of survival and fecundity has its maximum value. It is calculated to be 26.3°C. We assume that such warm places will always be available in the stable, either on the walls and ceiling or on the animals. Immature parasitoids are exposed to the same temperatures as the fly pupae, and so are the adult parasitoids because they spend time in the manure searching for pupae.

3.14. Estimation of parameters

The submodels describing temperature-dependent development, survival, hatching and fecundity for each species and each life stage are based on the same mathematical equation called the SANDY model (Nachman & Gotoh 2015). This equation contains 5 parameters, but in practice not all parameters have to be estimated. As demonstrated in Appendix 2 we were able to use empirical data to estimate all the parameters needed to parameterize development, survival, hatching and fecundity rates. However, these estimates are all obtained from laboratory experiments where starvation and competition were minimized. We lacked laboratory data to parameterize the parameter determining how competition among fly larvae affects their survival and two parameters describing how temperature varies in the manure in response to air temperature. The missing parameters were instead obtained by calibrating the models to stable data (see Section 4.3. and 4.4).

3.15. The computer program

A simulation model, in contrast to an analytical model, calculates the state variables characterizing the state of the system at time *t* by means of an iterative process, where the state of the system at time *t* is calculated from its state at time *t*-1 using the functional relationships to calculate the system's changes during each time step (Fig. 3.2). To carry out such very time-requiring calculations, it is necessary to transform the mathematical description of the model into a computer program. The program used to simulate the fly-parasitoid interactions is named *The Fly Simulator* (see Appendix 3). It allows the user to initialize the system, i.e. defining its state variables at time 0, the parameter values to be used in the functional relationships, the size of the system (number and area of stable compartments), and the temperature regime that drives the system. During a simulation, the model's interface allows the user to follow the dynamics of the system and interactively interfere with the system by choosing among the following actions: Releasing parasitoids, applying a larvicide, removing manure or use a combination of these actions. The outcome of the chosen control strategy is measured in terms of *FlyDays*, which is the cumulated number of adult stable flies occurring in the stable from day 0 through day *t*. The efficiency of a given control strategy is assessed by expressing the decrease in *FlyDays* in percentage of the maximum *FlyDays*, i.e. when no control measures are taken.

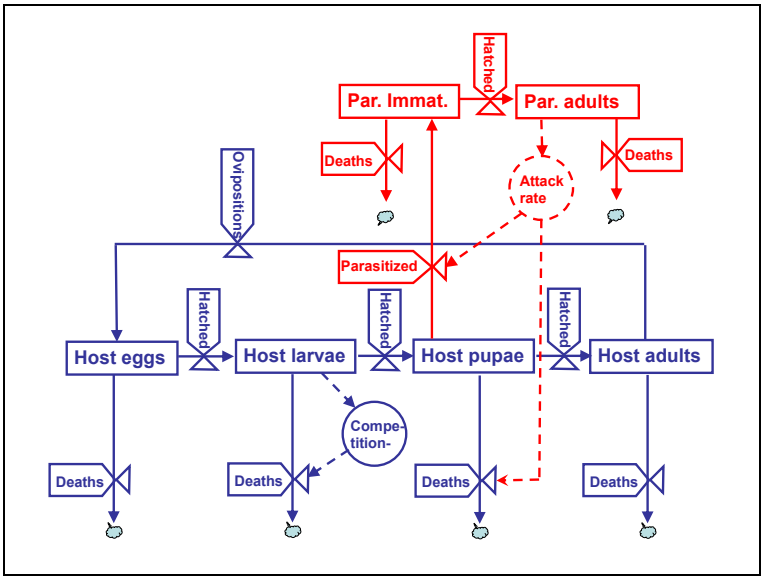

FIG. 3.2. FLOW DIAGRAM SHOWING THE MODELLED SYSTEM. RECTANGLES SHOW STATE VARIABLES (NUMBER OF INDIVIDUALS IN EACH LIFE STAGE). RATES ARE SHOWN WITH VALVE SYMBOLS. THEY DETERMINE THE SPEED AT WHICH INDIVIDUALS FLOW THROUGH THE SYSTEM (FULL LINES). CIRCLES INDICATE FEED BACK MECHANISMS IN THE SYSTEM.

3.16. Initializing the age distributions

The model not only needs to be initialized with respect to the number of individuals within each life stage, but also with respect to the age distribution within the stages. Two approaches can be taken: (*a*) All individuals in a stage have age 0 (i.e. they have just entered the stage) or (*b*) the age-distribution is stable. The former allows the user to simulate life-table experiments where the survival and reproduction of a *cohort* (i.e., a group of individuals born at the same day) is followed over time in a constant environment. A *stable age-distribution* is the age-distribution achieved when a population is allowed to grow in a constant environment without any *limiting factors* (i.e., food is supplied ad libitum, absence of enemies and diseases, no crowding effects).

4. Calibration and validation

4.1. Introduction

Evaluation of a model's quality typically involves two successive steps: (1) Does the model behave in a realistic way yielding predictions that are qualitatively in accordance with our expectations and common sense? (2) Does the model produce predictions that are quantitatively in agreement with empirical data? The first step is called *verification* and the last step *validation*. Verification takes place during the development of the model and the end result is the model's functional structure, i.e. its state variables and the equations describing the changes in these variables.

When a model has been through the verification process, its predictions depend on the numerical values of its parameters. By varying the parameter values, it may be possible to improve the quantitative agreement between predictions and observational data. This process is called parameter *calibration*. When a model has many parameters (as the present model has), the number of different combinations of values these parameters can take is enormous. It is therefore necessary to limit the calibration process to those parameters that have not been estimated from independent experiments. Fortunately, we have been able to estimate all parameters by means of independent experiments (see Appendix 2) except three, namely K, c and ψ. K is the parameter used to model heat production per time unit due to fermentation of manure and c is the parameter that models how fast heat is transferred through the manure by conduction. ψ relates the density of fly larvae to larval mortality and expresses the effect of intraspecific competition among fly larvae. The values of K, c and ψ were therefore obtained by calibration.

4.2. The modelled farm system

We used data from a cattle farm located in Marke, North-west Zealand (55°41'52.2"N,11°27'4.3"E) to examine the ability of the model to simulate the observed population dynamics of stable flies from March 1, 2003, through December 20, 2004 (Skovgård & Nachman 2012). The farm is an organic dairy farm with about 60-70 milking cows and 25-50 calves. The calves are raised in a separate building connected to the cow stable through two open doorways. It is the calf facility which is in focus of our modelling efforts, because stable flies are especially abundant here. The area of the calf building is approximately 300 m² of which about 144 m² are taken up by two separate compartments, each 3 m x 24 m, at each side of the building and separated by a 3.5 m broad gangway. The compartments can be further divided into separate sections of various sizes (min 3 m x 3 m). The calves are free-roaming within the sections where they are provided with straw-beddings. The manure thus consists of a mixture of straw, decomposing feed, faeces and urine. Removal of manure takes place at irregular intervals and not necessarily at the same time in both compartments. For simplicity we assume that the depth of manure is the same everywhere within a compartment and that 50 of the 72 m² constituting a compartment is covered with manure.

The start date of the model was set to January 1, 2003, so the period from the start date and until March 1, 2003, when the flies were sampled for the first time, can be considered as the initialization period allowing the populations to adjust to physical conditions inside the stable.

4.3. Calibration of manure temperatures

Air temperature inside the stable was measured during the experimental period yielding daily average temperatures from March 1, 2003, December 31, 2004. To obtain temperatures from January 1 through 28 February we used outside temperatures obtained from a nearby weather station owned by The Danish Meteorological Institute (DMI) and used a linear model to convert the outdoor temperature on a given day to the inside air temperature (see Section A2.4.1).

As manure temperature was not measured during the experimental period, we conducted temperature measurements at two later occasions: September 6, 2013, and December 5, 2013. The parameter values of the manure model were obtained by calibrating c and K until the best fit between measured and predicted manure temperatures was obtained (see section

A2.4.2 in Appendix 2). Figure 4.1 shows that the predicted temperature amplitude at four different depths decreases with depth, while the delay relative to air temperature increases with depth. The highest temperatures are predicted to occur at intermediate depths (10-15 cm), where favourable temperatures for development and survival of immature stages of flies and parasitoids are available all year around.

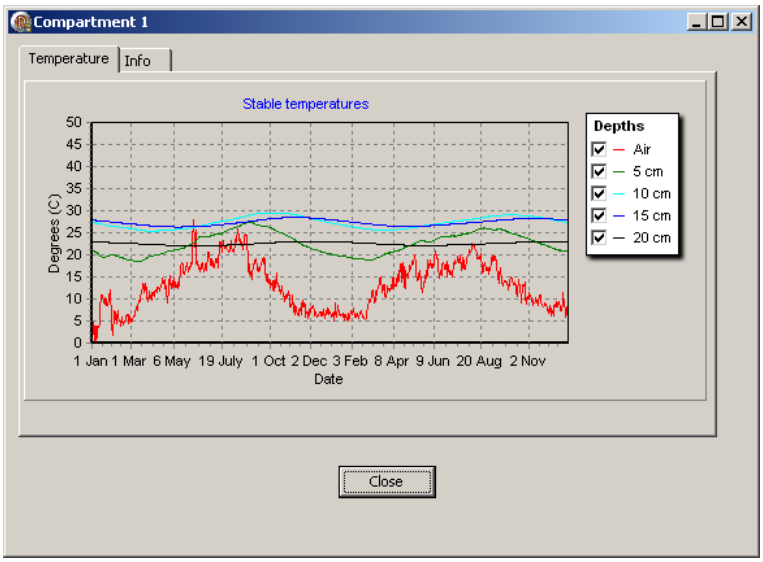

FIG. 4.1. PREDICTED MANURE TEMPERATURES IN 4 DIFFERENT DEPTHS BASED ON AIR TEMPERATURES IN MARKE. THE DEPTH OF THE MANURE IS SET TO BE 30 CM AND THE FLOOR TEMPERATURE TO BE 12.97^{0}C. PARAMETER VALUES OF EQN A1.25 ARE GIVEN IN TABLE A2.6.

4.4. Calibration of the larval competition coefficient

We used sampling data from Marke to calibrate the larval competition coefficient (ψ). Numbers of adult stable flies were estimated by means of a mark-recapture method at 22 sampling occasions from March 2003 through December 2004 with monthly intervals (Skovgård & Nachman 2012). Figure 4.2 shows the sampling data together with the predicted dynamics based on the simulation model and the parameters obtained from laboratory experiments and calibration (see Section A2.5 in Appendix 2).

Because the stage distribution of stable flies on January 1, 2003, is unknown, we assumed that it was the same as on January 1, 2005. We applied an iterative procedure (see Appendix 2) to estimate the initial population to consist of 636,193 stable flies of which 235,562 were eggs (37.0%), 288,712 larvae (45.4%), 103,458 pupae (16.3%) and 8461 adults (1.3%). We also assumed that parasitoids were absent (Fig. 4.3). As no information about manure depth is available, we assumed that the area covered with manure was 100 m^2 with an average depth of 20 cm and that the amount of manure did not change with time. A stable age distribution was applied to initialize the model with respect to the age distribution of individuals within each life stage.

ψ was changed in steps of one, and for each value of ψ the fit of the model to sampling data was expressed by the sum of squared deviations. The best fit was achieved when ψ = 20 cm^3/larvae. The model explains about 64% of the total variation in the observed numbers of adult stable flies. In the following we will refer to the simulation shown in Fig. 4.2 as the standard scenario against which the other runs can be compared.

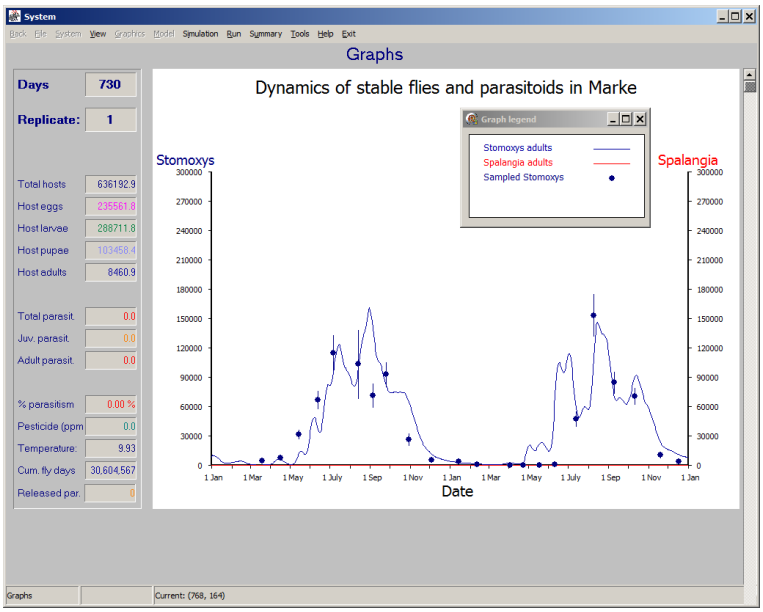

FIG. 4.2. OBSERVED ABUNDANCE OF ADULT STABLE FLIES IN MARKE IN 2003 AND 2004 (DOTS AND 95% CONFIDENCE INTERVALS) AND THE PREDICTED DYNAMICS (LINE) USING THE PARAMETER VALUES GIVEN IN TABLES A2.3-A2.6. MANURE DEPTH WAS ASSUMED TO BE 20 CM. THE RUN REPRESENTS THE STANDARD SCENARIO.

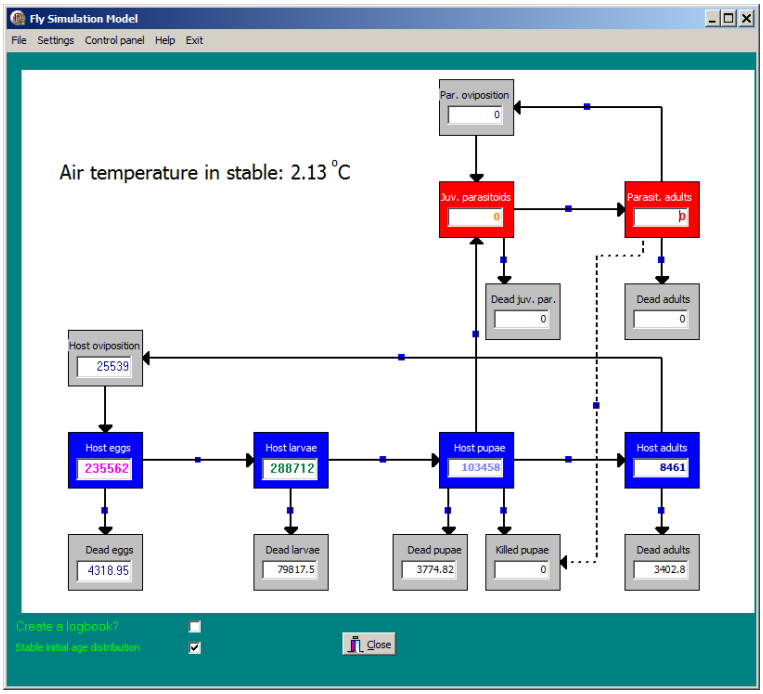

FIG. 4.3. INITIAL STAGE DISTRIBUTION USED TO SIMULATE THE DYNAMICS OF STABLE FLIES IN THE STABLE IN MARKE (FIG. 4.2). THE SIMULATION STARTS AT JANUARY 1, 2003, WHEN THE AIR TEMPERATURE INSIDE THE STABLE WAS 2.13°C.

5. Analyses of the system

5.1. Purposes and general procedure

In this section we will investigate the internal dynamics of the simulation model in a constant environment. We assume that everything is the same as in the standard scenario except that we set the temperature to be constant. It means that the properties of the modelled system will appear more clearly because the dynamics is not overridden by seasonal and random variations in temperature. We will further assume that all life stages are exposed to the same temperature, implying that the temperature in the manure is the same at all depths and the same as in the air. The purpose is first to identify the temperature interval allowing the stable fly population to persist in absence of parasitoids. We will then introduce 20,000 parasitoids (10,000 immatures and 10,000 adults) into the system to investigate at which temperatures the parasitoid population can coexist with the stable flies and to reveal the type of underlying dynamics, e.g. damped oscillations, stable limit cycles or chaotic fluctuations (May 1974).

5.2. Dynamics of stable flies without parasitoids

The simulations demonstrate that a population of the stable flies at temperatures in the range between13.1°C and 33.8°C and in absence of parasitoids will approach an equilibrium steady state through damped oscillations. The time until equilibrium is reached and the size of the equilibrium population depend on temperature (Fig. 5.1). The highest equilibrium population size is achieved at about 28°C (Fig. 5.2). At this temperature, the stable age distribution is characterized by 42.3% eggs, 44.9% larvae, 5.6 % pupae and 7.2% adults.

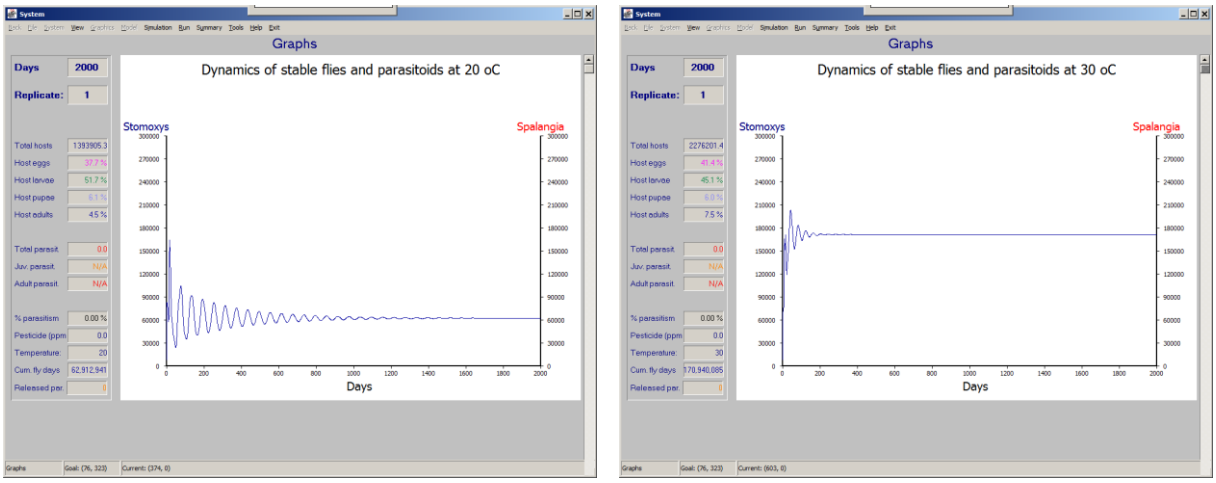

FIG. 5.1. DYNAMICS OF ADULT STABLE FLIES AT TWO DIFFERENT TEMPERATURES AND IN ABSENCE OF PARASITOIDS. MANURE DEPTH IS 20 CM.

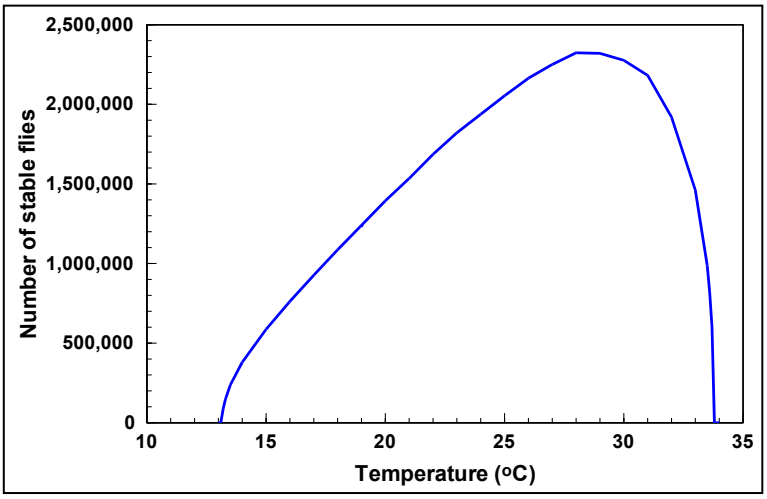

FIG. 5.2. THE RELATIONSHIP BETWEEN TEMPERATURE AND THE EQUILIBRIUM POPULATION SIZE (ALL STAGES COMBINED) OF STABLE FLIES WHEN THE DEPTH OF MANURE IS 20 CM.

We also investigated under which conditions the dynamics of stable fly populations may become oscillatory, as observed in Nicholson's (1954) experiments with blowflies (*Lucilia cuprina* Wied.). As seen from Fig. 5.3., increasing the maximum fecundity rate (F_{max}) from the value used in the simulation model (i.e. F_{max} = 11.86 eggs d^{-1}) to 16 eggs d^{-1} can change the dynamics of stable flies from damped oscillations to stable limits cycles, but only in the temperature range between 19.8°C and 25.3°C. The higher the value of F_{max}, the broader the temperature range for stable limit cycles.

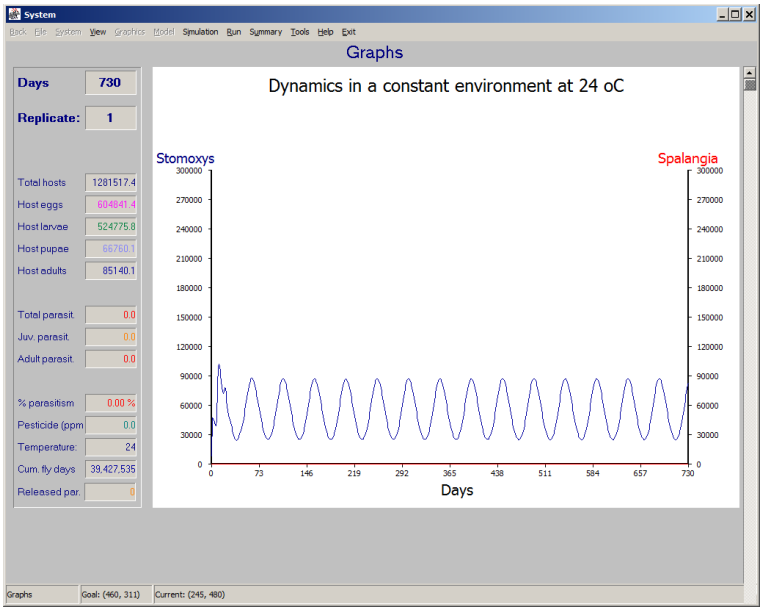

FIG. 5.3. STABLE LIMIT CYCLES GENERATED BY THE MODEL IF THE MAXIMUM FECUNDITY RATE INCREASES FROM 11.86 TO 16 EGGS PER FEMALE PER DAY AND THE TEMPERATURE IS 24°C. ONLY NUMBER OF ADULT FLIES IS SHOWN.

5.3 Dynamics of stable flies and parasitoids

The simulations show that *S. cameroni* requires temperatures between 22.9°C and 32.4°C in order to coexist with stable flies. The dynamics of the system becomes more complex when both species are present than when stable flies occur alone (Fig. 5.4). As long as the temperature is between 22.9°C and 24.5°C, the system exhibits damped oscillations towards constant population sizes at equilibrium. At 24.5°C the system has a bifurcation point where the dynamics changes to exhibit stable limit cycles (i.e. cycles that repeat themselves with a constant period and amplitude). This type of dynamics prevails until 31.9°C, when the system returns to damped oscillations (Fig. 5.5).

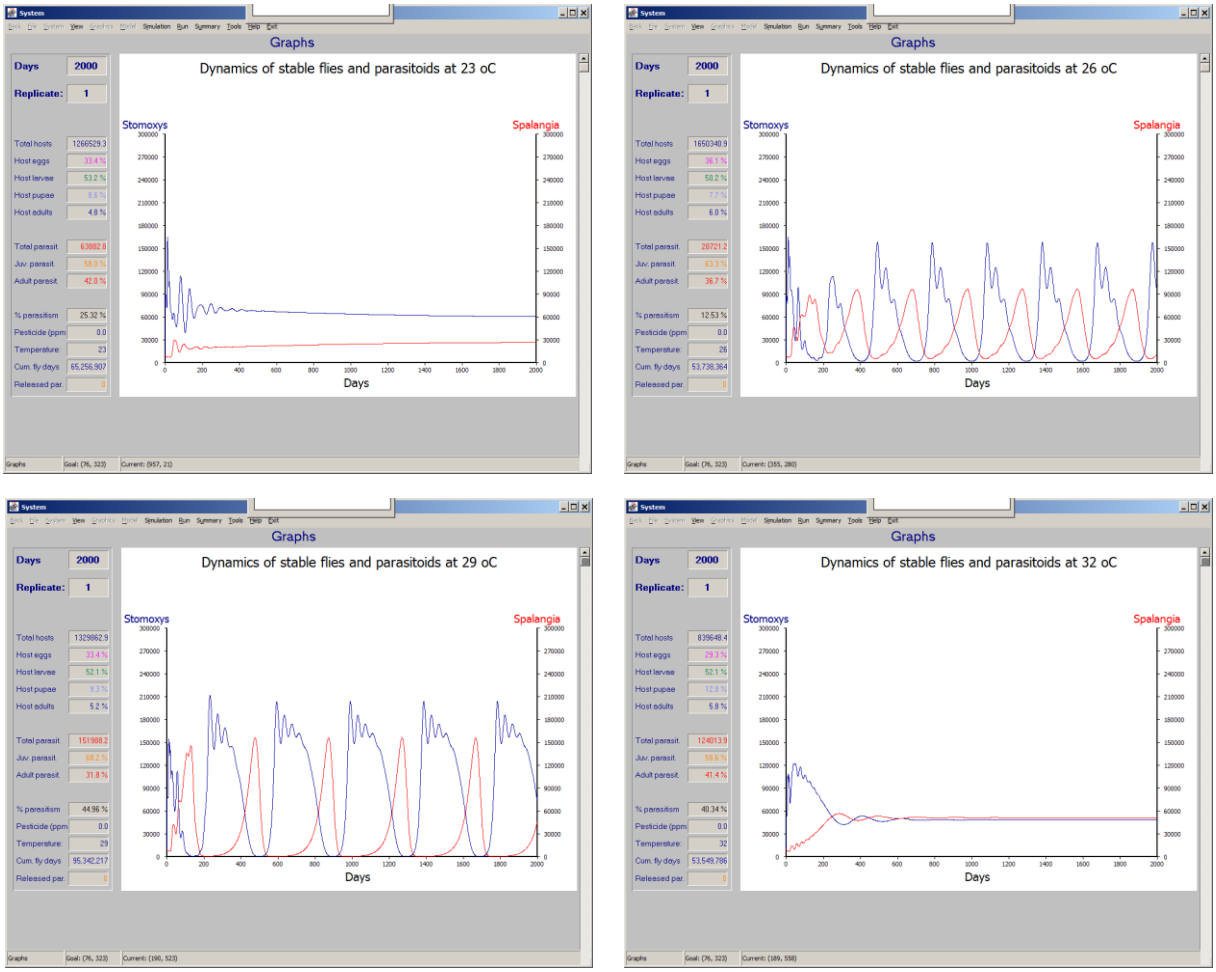

FIG. 5.4. DYNAMICS OF ADULT STABLE FLIES AND PARASITOIDS AT FOUR DIFFERENT TEMPERATURES SHOWING THE SHIFTS IN DYNAMICS (DAMPED OSCILLATIONS AT 23^{o}C AND 32^{o}C AND STABLE LIMIT CYCLES AT 26^{o}C AND 29^{o}C).

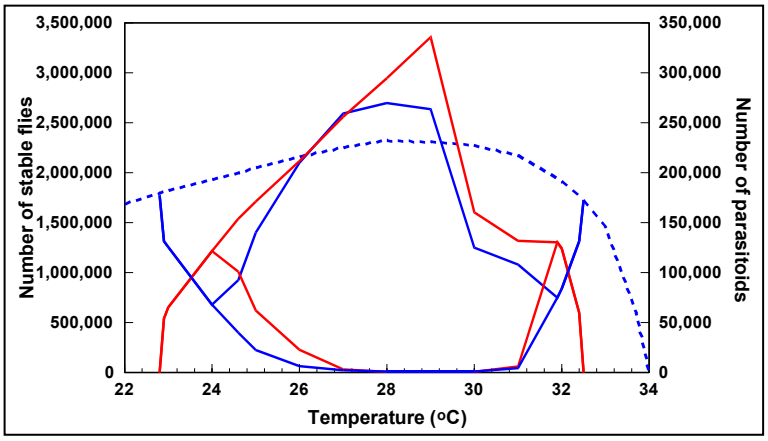

FIG. 5.5. BIFURCATION DIAGRAM SHOWING THE EFFECT OF TEMPERATURE ON THE DYNAMICS OF STABLE FLIES (BLUE LINES) AND PARASITOIDS (RED LINES). FULL LINES SHOW THE DYNAMICS WHEN BOTH SPECIES ARE PRESENT WHILE BROKEN LINE SHOWS THE EQUILIBRIUM POPULATION SIZE OF STABLE FLIES WITHOUT PARASITOIDS (SAME AS FIG. 5.2). THE FULL LINES SPLIT INTO TWO BRANCHES WHEN THE TEMPERATURE IS BETWEEN 24.5^{o}C AND 31.9^{o}C BECAUSE THE DYNAMICS CHANGES FROM DAMPED OSCILLATIONS TO STABLE LIMIT CYCLES. THE LOWER AND UPPER BRANCHES MARK THE MINIMUM AND MAXIMUM POPULATION SIZES OF EACH SPECIES DURING A CYCLE.

6. Sensitivity analysis

6.1. Purpose and procedure

The parameters and assumptions of any model are subject to change and error. Sensitivity analysis is the investigation of these potential changes and errors and their impacts on conclusions to be drawn by the model (Pannell 1997). In the following, we apply *elasticity*, defined as (Pannell 1997)

$$e = \left(\frac{\partial Y}{\partial X} \right) \left(\frac{X}{Y} \right) \tag{6.1}$$

to investigate how sensitive the model predictions are to changes in parameter values. Thus, let Y denote the number of *FlyDays* obtained from the standard scenario with parameter values given in Tables A2.3-A2.6 and X the standard value of a given parameter. $\partial Y/\partial X$ is the slope of the relationship between X and Y, estimated as $\partial Y/\partial X \approx \Delta Y/\Delta X$, where ΔY is the change in Y when the parameter value changes from X_{min} to X_{max}. In most cases, we used $\Delta X = \pm 0.1X$, i.e. $X_{min} = X - 0.1X$ and $X_{max} = X + 0.1X$, but in cases where the standard value of X is 0, we applied $X_{min} = 0$ and $X_{max} = 0.1$, and set X to 0.05. We also modified ΔX in cases where the parameter in focus is a probability bound to be 0 and 1. In such cases, we set X_{min} to 0 if $X - 0.1X < 0$ and to X_{max} to 1 if $X + 0.1X > 1$. Finally, if a parameter is bound to take integer values, X_{min} is rounded to the largest integer value satisfying $X_{min} \leq X - 0.1X$ and X_{max} rounded to the smallest integer value satisfying $X_{max} \geq X + 0.1X$.

Elasticity was calculated for all parameters in the host-parasitoid model except for the structural parameters. A structural parameter is one which is by definition is set to 0, either because the underlying functional relationship is assumed to be independent of temperature (e.g. sex ratio) or to be monotonically increasing with biological age (e.g. hatch rate). Thus, sensitivity analysis involved 97 parameters. To limit the number of simulations, we changed the parameters singly while keeping all the other parameters at their standard values, resulting in 194 runs of the model. It has to be emphasized that the sensitivity analysis conducted in this report is simplified and preliminary as it does not include interactions when two or more parameters vary simultaneously (see e.g. Clemson et al. (1995) for more sophisticated approaches to sensitivity analysis).

As the standard scenario we applied the same initial populations of stable flies and parasitoids as in Section 5.3. However, instead of keeping temperature constant, we subjected the system to environmental stochasticity by adding white (uncorrelated) noise to the temperature. Thus, it was assumed that the daily ambient temperature could vary stochastically between 15°C and 35°C around a mean temperature of 25°C. To achieve this, a random daily temperature was generated as $T = 15°C + u \cdot 20°C$, where u is a standardized evenly distributed pseudo-random variable that takes values between 0 and 1. The sequence of daily temperatures was the same for all parameters.

The numerical value of elasticity indicates how sensitive the model is to variation in a given parameter. A positive value of e indicates that *FlyDays* will increase with a higher parameter value, while the opposite applies for a negative value.

We also tested to what extent sensitivity of a given parameter depends on the sequence of daily temperature by conducting ten replicates differing only with respect to the stochastic sequence of temperatures, enabling us to calculate the average and standard error of the ten elasticity values.

6.2.

Results

Sensitivities of all parameters occurring in the parasitoid-host model are shown in Tables A2.3-A2.6 in Section A2.6. Fourteen parameters show elasticity values that numerically exceed 1 (6 positive and 8 negative). Eight of these parameters are associated with *S. calcitrans*, 3 with *S. cameroni*, 2 with the interactions between the two species, and one with the conditions prevailing in the manure. The most sensitive parameters for *S. calcitrans* are associated with maximum daily survival rate (s_{max}) of larvae (e = 4.977), pupae (e = 4.409) and adults (e = 2.010), demonstrating that improved survival of the stable flies enhances the pest problem, while fewer flies are expected if T_{max} (e = -2.750), Q_{max} (e = -2.181) or the competition coefficient (ψ) (e = -1.026) of the larval stage increases. The same applies if Q_{max} of the pupal stage increases (e = -1.333).

For *S. cameroni*, the most sensitive parameters were associated with maximum daily survival (s_{max}) of immatures (e = -8.565) and adults (e = 2.768). Thus, improved survival of immature parasitoids is expected to decrease the fly problem, whereas the opposite applies to survival of the adults. With respect to the immature stages, a 10% reduction in daily survival rate is enough to drive the parasitoid population to extinction. The parasitoid's preference for host pupae of a given age (Eqn A2.15) was found to be sensitive to the parameter a (e = -1.299), indicating that the ability of *S. cameroni* to control *S. calcitrans* increases, the more selective the parasitoids are against a given age group of host pupae. Figures 6.1-6.3 show the model output for the three most sensitive parameters (s_{max} for larvae and pupae of *S. calcitrans* and immatures of *S. cameroni*).

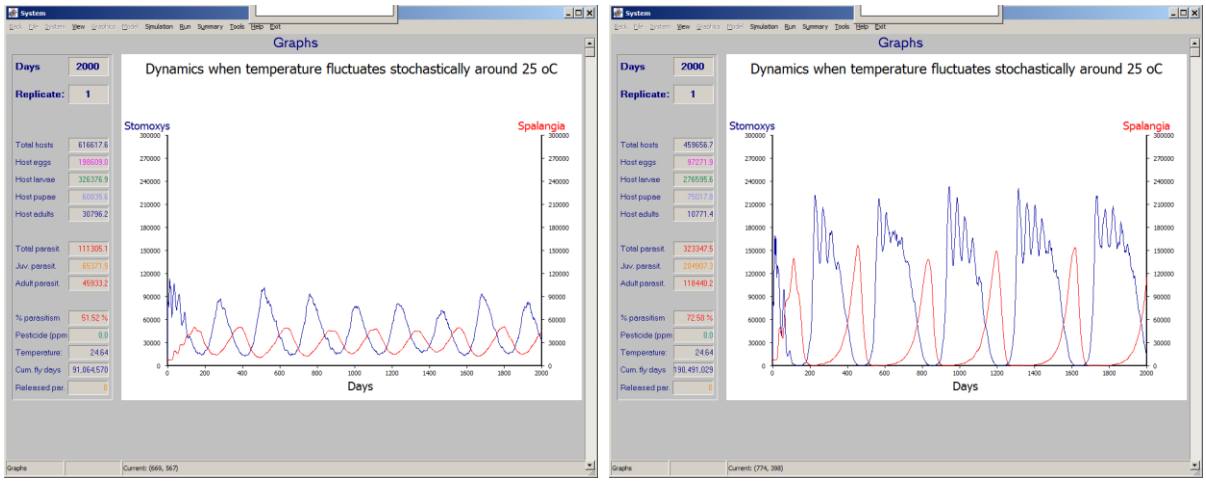

FIG 6.1. PREDICTED DYNAMICS WHEN LARVAL SURVIVAL (S_{MAX}) OF *S. CALCITRANS* IS REDUCED TO 0.8897 DAY^{-1} (LEFT) AND INCREASED TO 1.0 DAY^{-1} (RIGHT) .

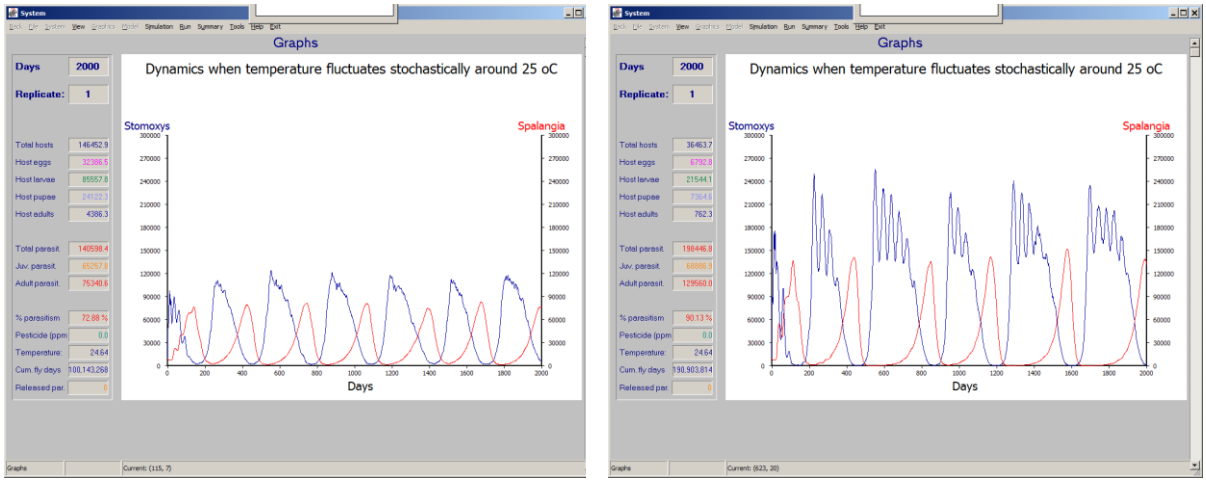

FIG 6.2. PREDICTED DYNAMICS WHEN PUPAL SURVIVAL (S_{MAX}) OF *S. CALCITRANS* IS REDUCED TO 0.8868 DAY^{-1} (LEFT) AND INCREASED TO 1.0 DAY^{-1} (RIGHT).

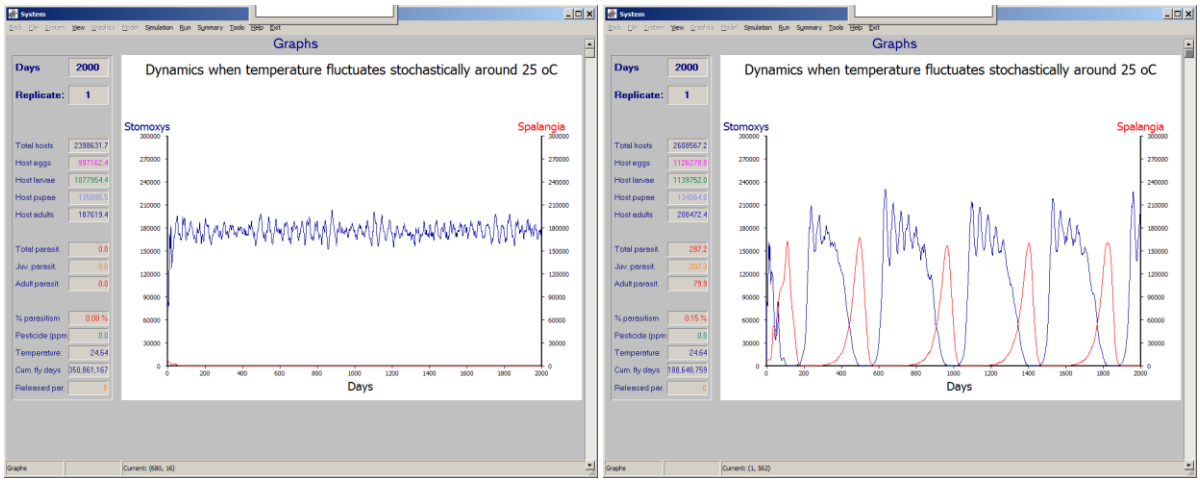

FIG 6.3. PREDICTED DYNAMICS WHEN IMMATURE SURVIVAL (S_{MAX}) OF *S. CAMERONI* IS REDUCED TO 0.8949 DAY^{-1} (LEFT) AND INCREASED TO 1.0 DAY^{-1} (RIGHT).

Neither ψ nor L_2 have been estimated from independent experiments and are therefore considered to be more uncertain than parameters obtained from experiments. We therefore selected these two parameters for investigating to what extent their elasticity values depend on the random sequence of daily temperatures. For ψ, the average of e based on 10 random temperature sequences was found to be -0.821 (SE = 0.311). Assuming that e is normally distributed, the 95% confidence limits are between -1.525 and -0.117. For L_2 the average was 3.034 (SE = 1.097) and the 95% confidence limits to extend from 0.552 to 5.515. Although these results do not invalidate the conclusions based on a single temperature sequence, they nevertheless show that variability among individual replicates has to be taken into consideration if elasticities are estimated by means of the method used in this report.

7. Control strategies

7.1. Purposes and general procedure

The purpose of the analyses is to apply the standard scenario based on data obtained in a dairy cattle operation in Marke (Fig. 4.2) to investigate the efficacy of *S. cameroni* to control stable flies. We compare different release strategies in order to optimize the timing of releases and the number of released parasitoids. The abundance of adult stable flies occurring in the stable is expressed as *FlyDays* (*FD*) (see Section 3.15). The effect of a control measure is expressed by means of a control index (*CI*), which is the percentage reduction in *FlyDays* achieved by the chosen control strategy. The index is calculated as $CI = 100\left(\frac{FD_0 - FD}{FD_0}\right)\%$, where *FD* and FD_0 denote *FlyDays* with and without control measures. Figure 4.2 shows that *FlyDays* in Marke summed up to 30,604,567 or ca 42,000 flies as a daily average.

Though biological control is the main objective of this project, the model also includes options for chemical (application of larvicide) and physical control (removal of manure). We run simulations where these control methods were used either singly or in combination with the other methods. However, it should be emphasized that the comparisons of control methods are very tentative and carried out mainly for illustrative purposes to demonstrate the potential of applying an integrated pest management (IPM) approach.

Because the main reason for controlling stable flies in Denmark is to avoid the nuisance exerted by this pest, thereby improving the comfort of the farmer and his animals, and because we have no realistic knowledge of the costs (in terms of money) of the various control methods, we are unable to carry out a proper cost-benefit analysis in order to identify the most cost-efficient method, i.e. the method that gives the best results at the lowest expenses. Instead we investigate the percentage reduction in *FlyDays* obtained by a given effort. However, despite of proper knowledge of the costs associated with the various control methods, we have nevertheless included an option to include costs in the simulation program called *The Fly Simulator* (see later).

7.2. Biological control by means of parasitoids

7.2.1. Release strategies

Biological control strategies based on natural enemies (i.e. predators, parasitoids or parasites) are usually categorized as *importation* (sometimes called *classical biological control*), *conservation* and *augmentation*. The latter can be divided in *inoculative* and *inundative* releases (see also Section 2.1.6).

Inundative releases can be used as part of a *pre-planned release strategy* where the number and timing of releases are decided prior to the season and independently of how the pest develops. An alternative to pre-planned releases is to apply an *adaptive release strategy* where the timing and/or the number of released parasitoids are decided as the season progresses and the current abundance of the pest is known. An adaptive release strategy is based on defining a *treatment threshold*, i.e. when the number of adult stable flies exceeds a certain density or index of abundance (see Section 2.1.4). As the parasitoids attack the pupal stage of the flies, treating the stable with parasitoids does not reduce the abundance of adult flies immediately. In fact, the abundance of adult stable flies may continue to increase for a while due to continued emergence of unparasitized pupae. However, when recruitment of new adult flies ceases, the abundance of adult flies is likely to decrease steeply because their life-expectancy at high temperatures is short (see Section 2.1.2). Nevertheless, the response time from a release and until the desired effect has been achieved makes adaptive release strategies more complicated to use than implementation of pre-planned strategies. Furthermore, adaptive release strategies are based on assessments of current pest density. To reduce sampling time it may be necessary to apply sampling methods based on indices (see Section 2.1.4), but such time-saving procedures are usually associated with considerable uncertainty, increasing the risk of making wrong decisions (i.e. release enemies when not needed or do not release when actually

needed). Another problem to be addressed when using an adaptive release strategy is the complicated logistics associated with ordering, requiring and releasing sufficient natural enemies when mostly needed. Thus, although adaptive release strategies in theory could be more cost efficient than pre-planned strategies, the extra costs and risks associated with implementation of the former may reverse the picture in favour of pre-planned releases.

An adaptive release strategy should not be confused with a tactical approach to pest control. The latter is based on combining available information on a given day in order to predict how the pest will develop during the following days or weeks depending on the current situation, the expected weather and the available intervention options, so as to guide the farm operator to choose the optimal decision at any time during a season. For this purpose, a simulation model is needed to process data and update the prognoses for the pest as time proceeds. In contrast, once a pre-planned or adaptive release strategy has been chosen, based on either practical experience or, as in this study, on a retrospective simulation study, the control strategy is based on simple rules of thumbs, which can be applied without access to computers (see also Section 2.2.5).

The analyses in Chapter 5 indicate that *S. cameroni* is a thermophile species that cannot survive during winter time in a Danish stable where the air temperature is often below 10°C for extended periods. This expectation was confirmed when the model was initialized with both species present (using 636,193 hosts and 20,000 parasitoids) and run with the temperatures measured in Marke. Biological control of stable flies should therefore be based on either inoculation or inundative releases. We assumed that the parasitoids were released shortly after they had emerged from incubated pupae, so that their age at the time of release was set to 0 days. In practice, however, newly emerged parasitoids can be stored for 4-5 days at 15°C prior to release (Skovgård 2004, 2006).

7.2.2. Inoculative releases

To compare different inoculative release strategies we conducted a series of simulations:

Series 1 aims at testing the possibility of using an inoculative release strategy. We used four different dates to inoculate the system: April 1, May 1, June 1 and July 1, and at each occasion 10,000 parasitoids (70.7% females) were released.

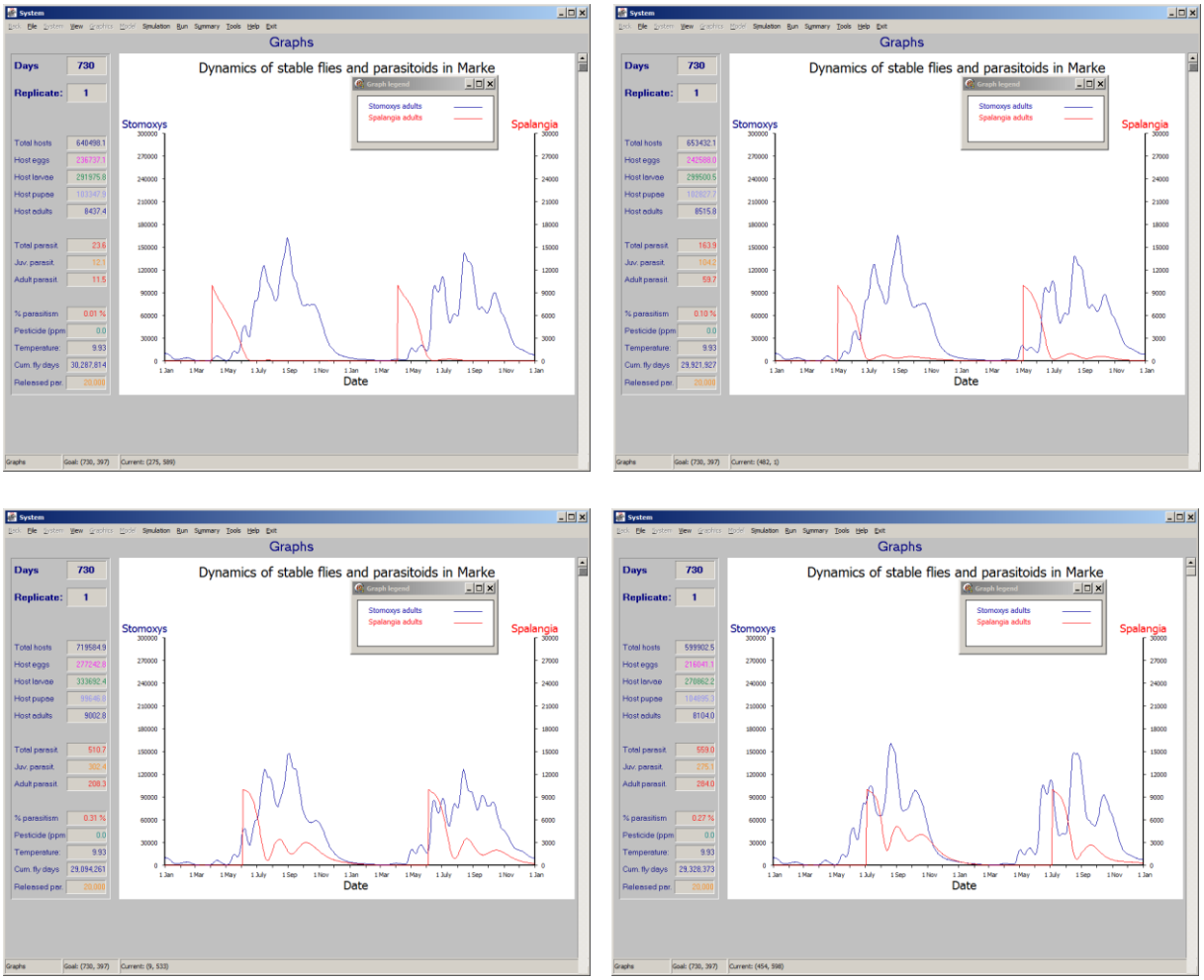

FIG. 7.1. OUTCOME OF SERIES 1. 10.000 PARASITOIDS WERE RELEASED ON APRIL 1 (UPPER LEFT GRAPH), MAY 1 (UPPER RIGTH GRAPH), JUNE 1 (LOWER LEFT GRAPH) AND JULY 1 (LOWER RIGHT GRAPH).

As seen from Fig. 7.1, the parasitoids went extinct if they were introduced too early in the season (April 1), whereas they persisted in the system until late autumn if they were released May 1 or later. However, the density of parasitoids remained low throughout the season and far from being sufficient to control the stable flies. Thus, the reductions in stable fly abundance (*CI*) are found to be 1.1% (April 1), 2.2% (May 1), 5.0% (June 1) and 4.2% (July 1). Consequently, this shows that an inoculative release strategy is inadequate to control stable flies. Instead we investigate whether the more costly inundative strategy can be applied.

7.2.3.

Preplanned inundative releases

Inundative releases involve two major costs: The cost of producing the parasitoids in the laboratory and the cost of transportation and releasing them in the stable. For a fixed number of parasitoids being released during a season, the total costs can be minimized if the number of releases can be reduced without jeopardizing control of stable flies. To investigate the relationship between number of releases (n), the number of released parasitoids per release (P) and the outcome of biological control, we conducted a series of simulations:

Series 2 aims at testing the effect of releasing parasitoids every t day from Jan. 1, 2003, through Dec. 31, 2004. We used the following release intervals: 7, 14, 21, 28, 42, 56 and 84 days, corresponding to n = 105, 53, 35, 27, 17, 14 and 9 releases. The total numbers of released parasitoids (P_{total}) were 500,000, 1 million and 2 million. The number of parasitoids used per release is found as $P = P_{total} / n$ rounded to nearest integer value. Series 2 comprises 21 individual runs. An example is shown in Fig. 7.2.

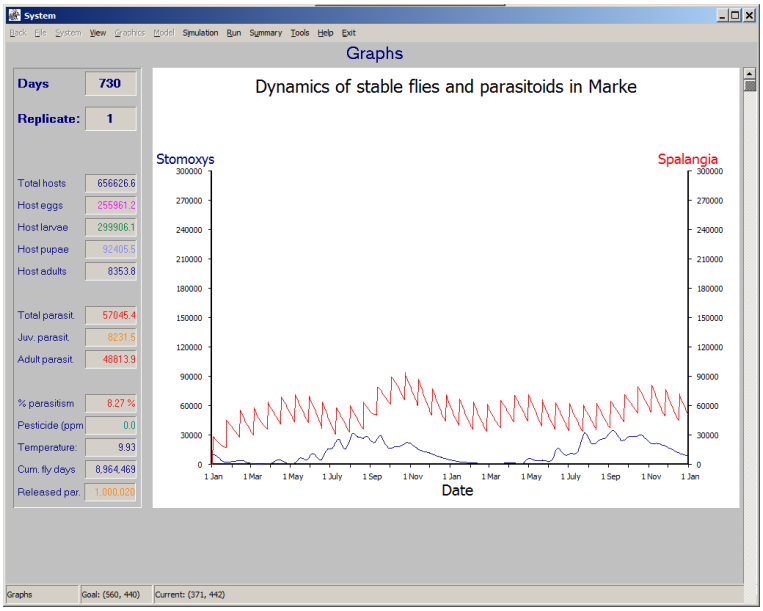

FIG. 7.2. A SAMPLE RUN FROM SERIES 2. 28571 PARASITOIDS WERE RELEASED EVERY THIRD WEEK FROM JAN. 1, 2003, THROUGH DEC. 31, 2004 (1 MILLION PARASITOIDS IN TOTAL). THE PARASITOIDS REDUCED THE NUMBER OF FLYDAYS TO 8,964,469, WHICH CORRESPONDS TO A CONTROL INDEX (*CI*) EQUAL TO 70.8%.

Figure 7.3 shows that the percent reduction in *FlyDays* (*CI*) increases with the total number of released parasitoids and decreases with the time interval between the releases, but not in a linear way. The control index is more or less the same as long as the frequency of releases does not exceed 4 weeks. Figure 7.3 also indicates that the gain per released parasitoid declines with the number released. Thus, releasing 18,519 parasitoids every 4 week gives an index of 42.3%. Doubling the number to 37,037 individuals increases *CI* to 69.1% while another doubling to 74,074 individuals results in an index of 97.3%. The relatively low efficiency per parasitoid when many parasitoids are released simultaneously is likely to be due to a decline in the functional response rather than to mutual interference, because the parasitoids cause a significant reduction in host density. Even if the release size is 74,074 parasitoids, the average parasitoid density is still low (< 0.1 cm^2). This density is below the density where mutual interference is likely to cause a significant reduction in attack efficiency (Fig. A2.18), in particular because about 30% of the released parasitoids are males.

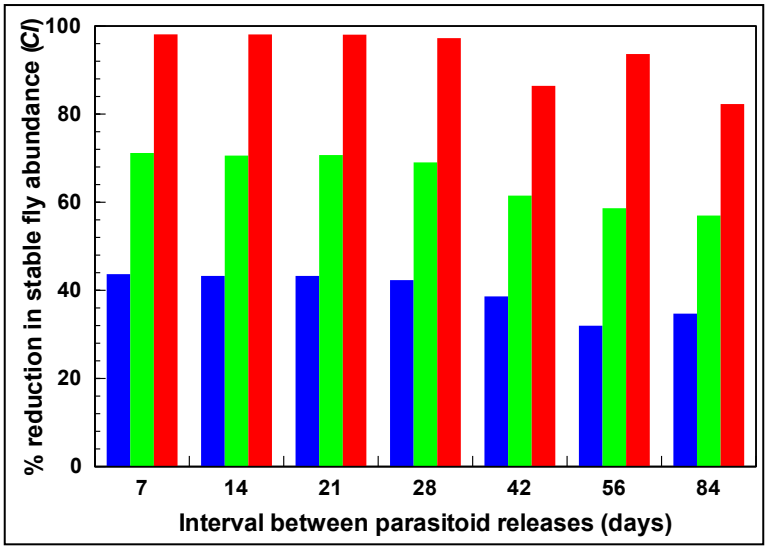

FIG. 7.3. RESULTS OF SERIES 2 SHOWING THE PERCENTAGE REDUCTION IN FLYDAYS (CONTROL INDEX) AS A RESULT OF THE NUMBER OF RELEASED PARASITOIDS AND THE INTERVAL BETWEEN RELEASES (BLUE BARS: TOTAL NUMBER OF PARASITOIDS IS 500,000; GREEN BARS: TOTAL NUMBER OF PARASITOIDS IS 1,000,000; RED BARS: TOTAL NUMBER OF PARASITOIDS IS 2,000,000).

Although Fig. 7.3 shows that inundative releases can significantly reduce stable fly abundances, it may be a waste of parasitoids and time to release them during winter time. To address this question we conducted a series of simulations:

Series 3 aims at testing whether parasitoids can be more efficiently used if their releases are synchronized with the periods where stable flies are most abundant, i.e. during spring, summer and autumn. We therefore run simulations where 250,000, 500,000 or 1 million parasitoids were released from April 15 through October 15 during the two years. Intervals between releases were 1, 2, 3 or 4 weeks, corresponding to 54, 28, 18, and 14 releases. Figure 7.4 shows a sample run.

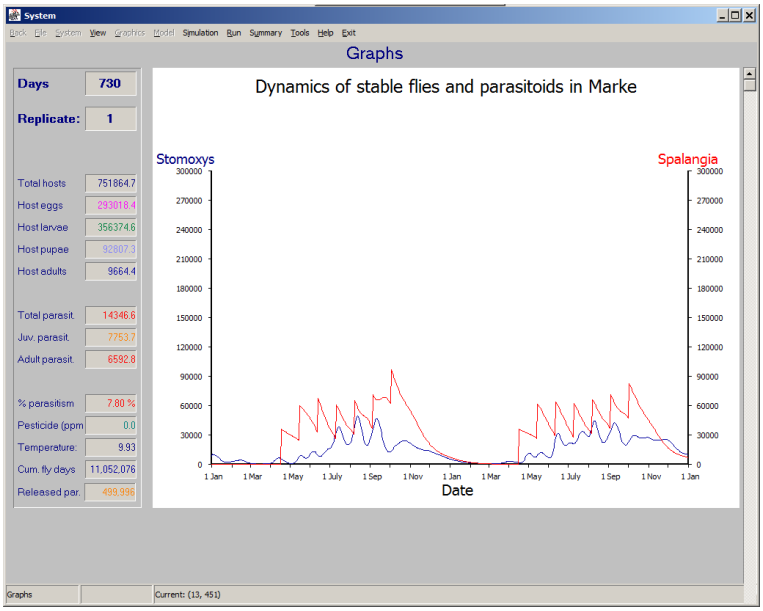

FIG. 7.4. A SAMPLE RUN FROM SERIES 3. 35,714 PARASITOIDS WERE RELEASED EVERY 4 WEEK FROM ARPIL 15 THROUGH OCTOBER 15 (500,000 PARASITOIDS IN TOTAL). THE CONTROL STRATEGY REDUCED THE STABLE FLY POPULATION WITH 63.9 %.

Figure 7.5 summarizes the results of the 12 runs in Series 3. It shows that confining releases of parasitoids to the period from April 15 to October 15 can effectively reduce the number of stable flies compared to the strategy where releases take place with fixed intervals throughout the entire season. For instance, releases taking place every four week during the stable fly seasons using 1 mill. parasitoids in total result in a control index of 96.0%, while the same number of released parasitoids only gives an index of 69.0% if they are released throughout the entire year. Figure 7.5 also shows that the interval between releases (max 4 weeks) has almost no influence on *CI* because longer intervals are balanced by larger release sizes. However, from a logistic point of view, it will be an advantage to minimize the number of releases.

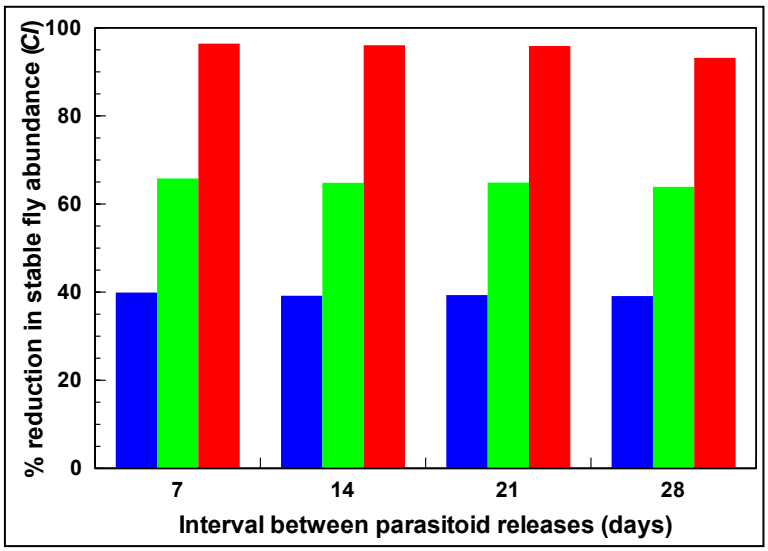

FIG. 7.5. RESULTS OF SERIES 3 SHOWING THE PERCENTAGE REDUCTION IN FLYDAYS (CONTROL INDEX) AS A RESULT OF THE NUMBER OF RELEASED PARASITOIDS AND THE INTERVAL BETWEEN RELEASES (BLUE BARS: TOTAL NUMBER OF RELEASED PARASITOIDS IS 250,000;

GREEN BARS: TOTAL NUMBER OF PARASITOIDS IS 500,000; RED BARS: TOTAL NUMBER OF PARASITOIDS IS 1,000,000). PARASITOIDS WERE ONLY RELEASED DURING THE PERIOD FROM APRIL 15 THROUGH OCTOBER 15.

7.2.4. Adaptive inundative releases

Series 1-3 are based on pre-planned releases, i.e. the releases take place independently of the abundance of stable flies, which may sometimes result in unnecessary releases. We therefore compare the pre-planned strategies with adaptive strategies, where parasitoids are released only if the abundance of stable flies exceeds a predefined threshold. Adaptive releases therefore require information about whether the threshold has been exceeded or not. In practice such information will be associated with considerable uncertainty, unless much time is invested in reliable sampling. However, in the following analyses we will ignore this uncertainty and instead make decisions based on the model predicted (true) population sizes.

Series 4 aims at testing strategies where interventions take place if the number of adult stable flies at a sampling occasion exceeds a threshold of either 10,000 or 20,000 individuals. The minimum time between releases was 1, 2, 3 or 4 weeks and the number of released parasitoids was 20,000 and 40,000 individuals/release when the threshold was 10,000 or 20,000 stable flies, respectively (Fig. 7.6).

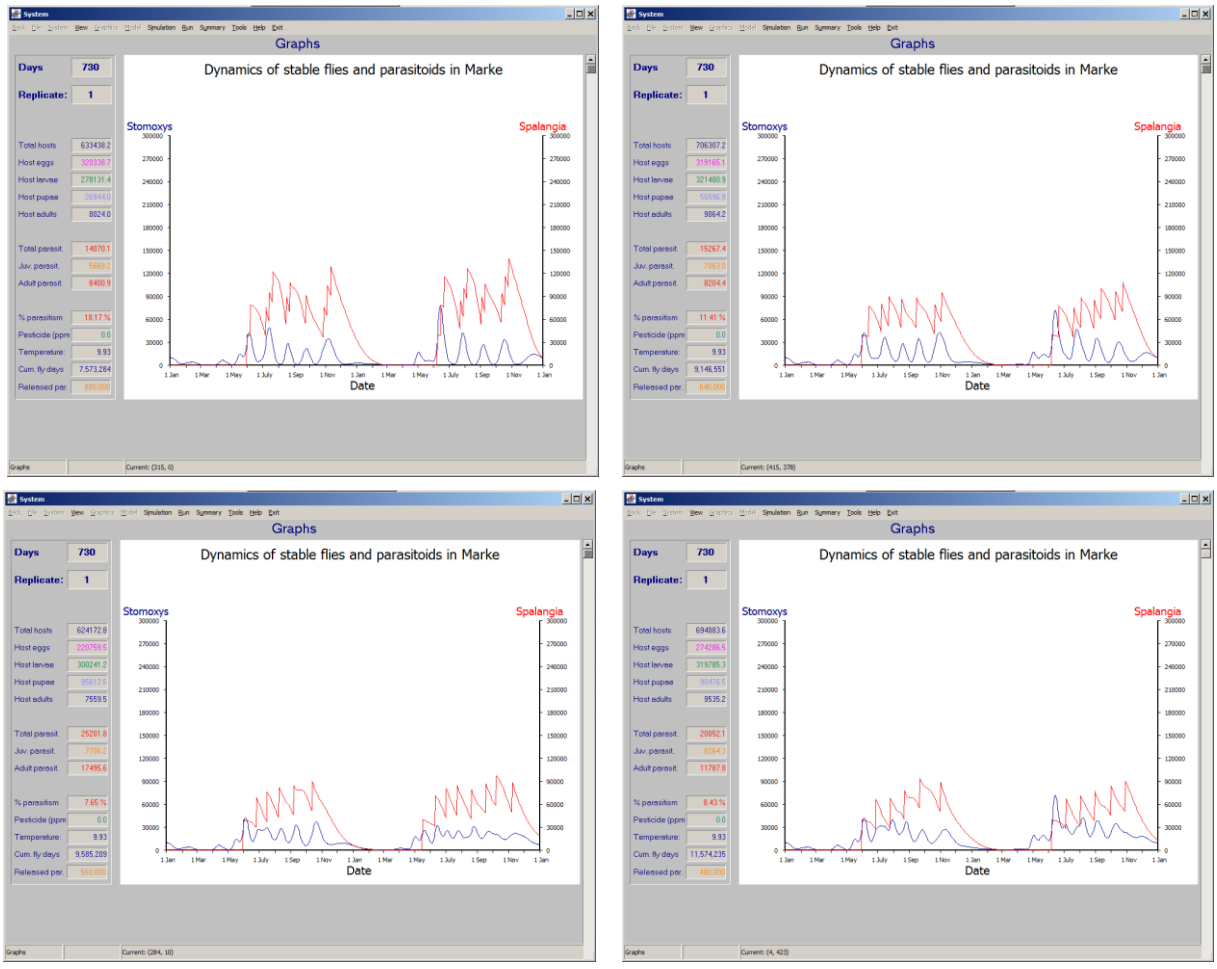

FIG. 7.6. RESULTS OF SERIES 4 WHERE 40,000 PARASITOIDS WERE RELEASED EACH TIME THE NUMBER OF ADULT STABLEFLIES EXCEEDED 20,000 INDIVIDUALS. THE MINIMUM INTERVAL BETWEEN TWO SUCCESSIVE RELEASES IS 1 WEEK (UPPER LEFT GRAPH), 2 WEEKS (UPPER RIGHT GRAPH), 3 WEEKS (LOWER LEFT GRAPH) OR 4 WEEKS (LOWER RIGHT GRAPH).

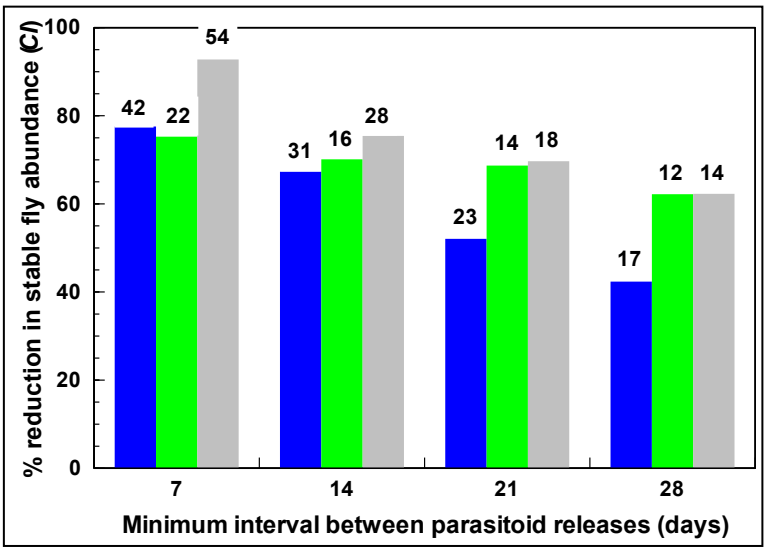

FIG. 7.7. RESULTS OF SERIES 4 BASED ON ADAPTIVE RELEASE STRATEGIES USING TWO DIFFERENT RELEASE THESHOLDS (BLUE BARS THRESHOLD IS 10,000 ADULT STABLE FLIES; 20,000 PARASITOIDS PER RELEASE. GREEN BARS: THRESHOLD IS 20,000 ADULT STABLE FLIES; 40,000 PARASITOIDS PER RELEASE). GREY BARS SHOW A PREPLANNED STRATEGY WHERE THE TOTAL NUMBER OF RELEASED PARASITOIDS IS THE SAME AS IN THE ADAPTIVE STRATEGY BASED ON A THRESHOLD OF 20,000 STABLE FLIES. THE NUMBER ON TOP OF EACH BAR SHOW THE NUMBER OF PARASITOID RELEASES.

An adaptive strategy based on releasing parasitoids every time the number of stable flies exceeds 10,000 adults yielded better results than a threshold of 20,000 individuals, but only when the time interval between releases is 1 week or longer (Fig. 7.7). However, when the time interval between releases is 2 weeks or more, the higher threshold value becomes equal to or better than the lower threshold. This becomes even more evident if the number of releases is taken into consideration, but at the expense of higher numbers of released parasitoids. Thus, if the interval between two releases is at least 4 weeks, it is possible to obtain a *CI* of 62.2% by means of 12 releases of 40,000 parasitoids each, whereas *CI* based on 17 releases of 20,000 parasitoids is only 42.4%. In the former case, the total number of parasitoids is 480,000 and in the latter 340,000. In comparison, a preplanned release strategy based on a total release size of 480,000 parasitoids produced a *CI* of 62.3% if 34,286 parasitoids were released every 4 week from April 15 through October 15 in both years (14 releases). Although this points at the adaptive strategy to be more cost efficient than the preplanned strategy, this is not likely to be the case when the cost of sampling and the risk of making wrong decisions based on uncertain information about population sizes are taken into account.

7.3. Chemical control

7.3.1. Application strategies

To investigate chemical control we assumed that flies were controlled by means of a larvicide. The effect of a larvicide depends on the concentration of the active substance in the manure and the specific dose-lethality relationship. The concentration of pesticide in the manure is affected by the amount of pesticide per unit volume, the frequency of applications and the rate of disappearance, which in turn is determined by the degradation rate and the rate at which manure is added and removed. To model chemical control, we used data for the larvicide azadirachtin, which is a growth inhibitor affecting moulting of larvae from one stage to the next and from the third and last larval stage to the pupal stage (Miller & Chamberlain 1989).

In the section we simulate chemical control by either applying it at fixed time-intervals independently of the current density of stable flies (i.e. a pre-planned application strategy) or when the current density exceeds a predefined threshold (i.e. an adaptive application strategy).

A given application strategy can be expressed by means of the cumulated pesticide dose (*CPD*), which is calculated as *CPD* = Dose*Applications. Dose is measured as the amount of active substance applied (in ml). For a given *CPD* the reduction in *FlyDays* is FD_0-*FD* (see Section 6.2.1). Since the reduction in *FlyDays* will be at the expense of the increased pesticide use, we calculate the gain:cost ratio as $G/C = (FD_0\text{-}FD)/CPD$ and use it as a criterion for comparing application strategies. Thus, the optimal strategy is the one that maximizes *G/C*.

7.3.2. Pre-planned application strategies

Similar to the pre-planned releases of parasitoids, we conduct simulations where larvicide is applied throughout the entire year or only through the fly season.

Series 5 aims on testing the relationship between the treatment intervals and the control index (*CI*). We applied four treatment intervals: 1, 2, 3 and 4 weeks. The amount of larvicide was adjusted to give average concentrations of 50, 100 or 150 ppm in the manure immediately after the application. These concentrations correspond to expected lethality rates of 99.04%, 99.83, and 99.94%, respectively.

Figure 7.8 shows a sample run from Series 5 and the control indices of the series are shown in Fig. 7.9.

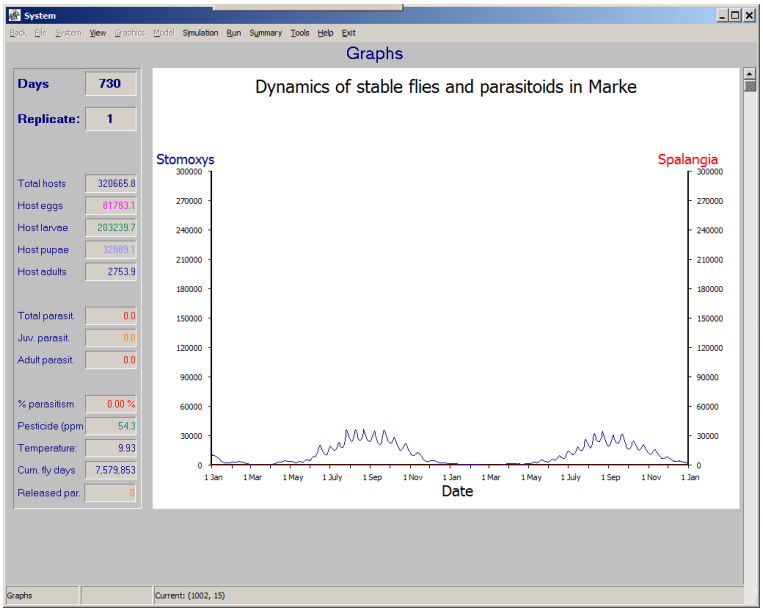

FIG. 7.8. A SAMPLE RUN FROM SERIES 5. PESTICIDE WAS APPLIED EVERY 2 WEEKS THROUGHOUT THE ENTIRE YEAR IN AN AMOUNT PER TREATMENT CORRESPONDING TO 50 PPM IN THE MANURE. THE CONTROL INDEX WAS 75.2%.

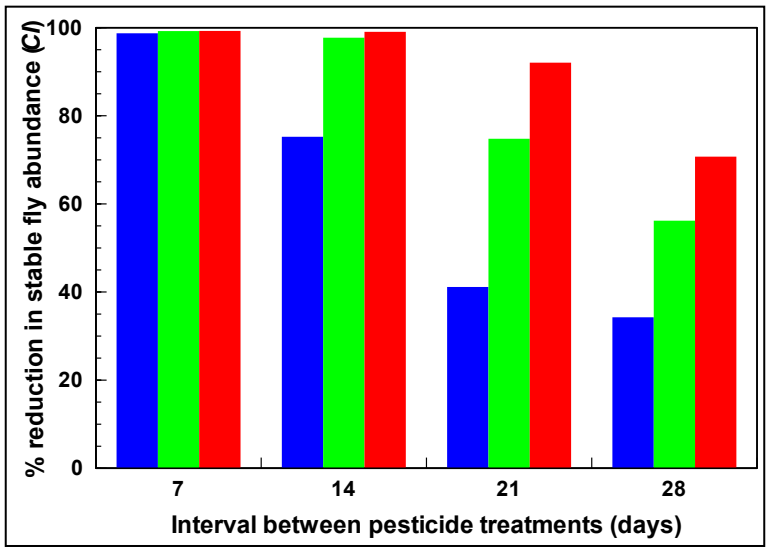

FIG. 7.9. RESULTS OF SERIES 5 WHERE THREE DIFFERENT DOSES OF PESTICIDES (BLUE BARS: 50 PPM; GREEN BARS: 100 PPM, RED BARS: 150 PPM) WERE APPLIED AT INTERVALS OF 1, 2, 3, OR 4 WEEKS THROUGHOUT THE ENTIRE YEAR.

Figure 7.9 shows that almost 100% success can be achieved when high doses of pesticide are applied with short intervals. The longer the intervals, the higher the dose to be applied in order to obtain the same success. However, using, e.g., a dose of 50 ppm every 2 weeks gives a better result than using 100 ppm every 4 weeks. It also shows that there is no advantage of applying high doses when treatments take place every week.

Series 6 aims at testing pre-planned application strategies during the fly season defined as the period from April 15 through October 15. Doses and intervals were the same as in Series 5.

Figure 7.10 shows that control of stable flies is only slightly poorer if use of pesticide is limited to the seasons where flies are usually most abundant. On the other hand, the number of applications is reduced by almost 50%.

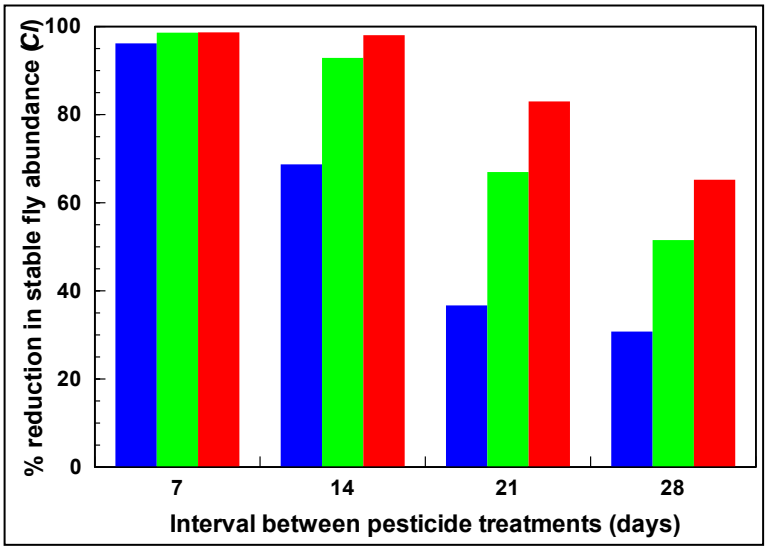

FIG. 7.10. RESULTS OF SERIES 6 WHERE THREE DIFFERENT DOSES OF PESTICIDES (BLUE BARS: 50 PPM; GREEN BARS: 100 PPM, RED BARS: 150 PPM WERE APPLIED AT INTERVALS OF 1, 2, 3, OR 4 WEEKS FROM APRIL 15 THROUGH OCTOBER 15 EVERY YEAR.

7.3.3. Adaptive application strategies

The adaptive application strategies are based on applying larvicide when the abundance of adult stable flies exceeds a given threshold value.

Series 7 examines the consequences of applying larvicide at three different concentrations (25, 50, 100 ppm) when the number of adult stable flies exceeds a threshold of 5,000 individuals.

Series 8 examines the consequences of applying larvicide at three different concentrations (25, 50, 100 ppm) when the number of adult stable flies exceeds a threshold of 10,000 individuals.

Series 9 examines the consequences of applying larvicide at three different concentrations (25, 50, 100 ppm) when the number of adult stable flies exceeds a threshold of 20,000 individuals.

As seen from Figs. 7.10 - 7.12, the best results in terms of *CI* were obtained when a threshold of 5,000 adult flies was used. The maximum value of *CI* is 79.7%, which was achieved when the dose was 100 ppm and the minimum time interval between treatments was 2 weeks. However, this required 28 treatments resulting in a *CPD* of 56,000 ml larvicide and a *G/C* ratio of 435.8. In comparison, the strategy based on using a threshold of 20,000 flies and 25 ppm pesticide gave a *G/C* ratio of 889.7 and a *CI* of 36.7% based on 25 applications (i.e. *CPD* = 12,500 ml). It makes this strategy the optimal one with respect to the balance between control and use of pesticides.

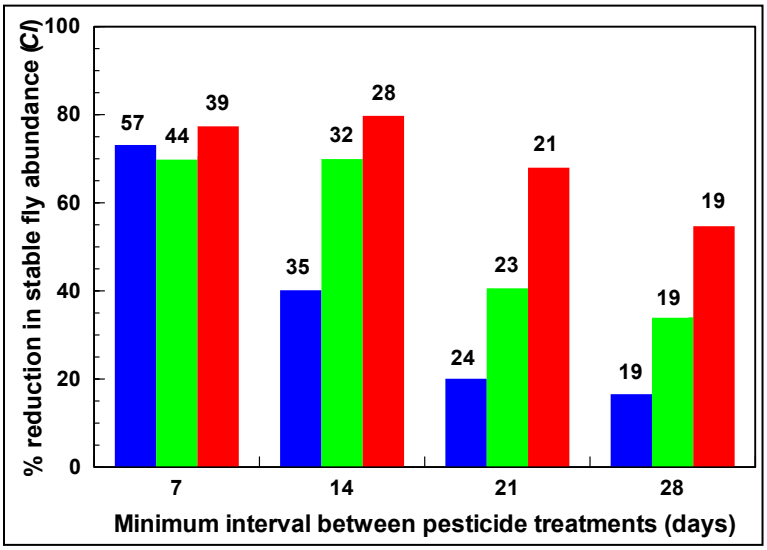

FIG. 7.11. RESULTS OF SERIES 7. LARVICIDE WAS APPLIED EACH TIME THE NUMBER OF ADULT STABLE FLIES EXCEEDED 5,000 INDIVIDUALS. MINIMUM INTERVALS BETWEEN TWO SUCCESSIVE APPLICATIONS WERE 1, 2, 3 OR 4 WEEKS, AND THE PESTICIDE DOSES USED WERE 25 PPM (BLUE BARS), 50 PPM (GREEN BARS) OR 100 PPM (RED BARS). NUMBER OF TREATMENTS IS SHOWN ON TOP OF BARS.

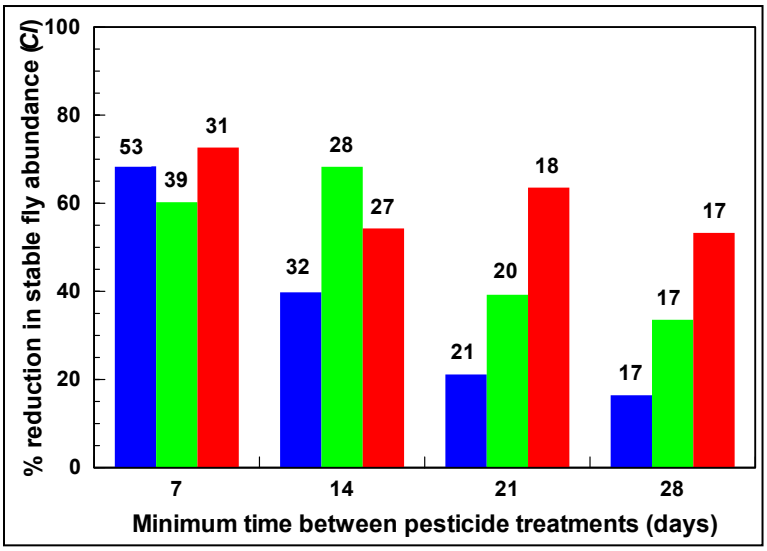

FIG. 7.12. RESULTS OF SERIES 8. LARVICIDE WAS APPLIED EACH TIME THE NUMBER OF ADULT STABLE FLIES EXCEEDED 10,000 INDIVIDUALS. MINIMUM INTERVALS BETWEEN TWO SUCCESSIVE APPLICATIONS WERE 1, 2, 3 OR 4 WEEKS, AND THE PESTICIDE DOSES USED WERE 25 PPM (BLUE BARS), 50 PPM (GREEN BARS) OR 100 PPM (RED BARS). NUMBER OF TREATMENTS IS SHOWN ON TOP OF BARS.

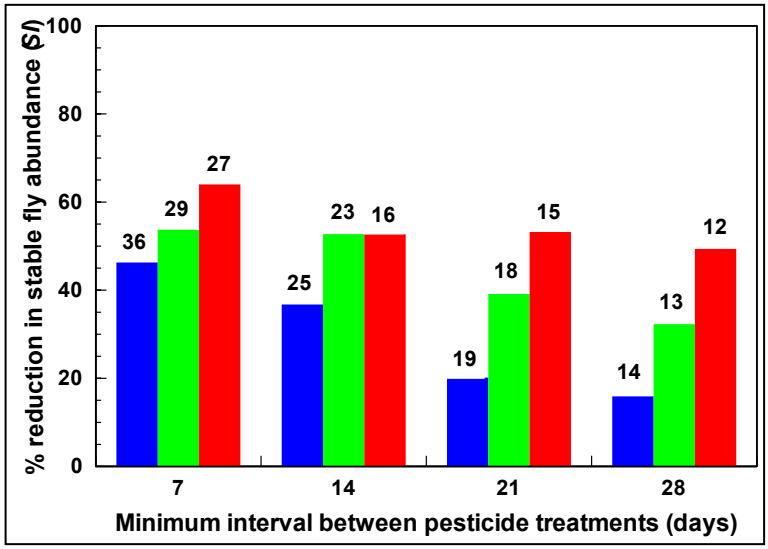

FIG. 7.13. RESULTS OF SERIES 9. PESTICIDE WAS APPLIED EACH TIME THE NUMBER OF ADULT STABLE FLIES EXCEEDED 20,000 INDIVIDUALS. MINIMUM INTERVALS BETWEEN TWO SUCCESSIVE APPLICATIONS WERE 1, 2, 3 OR 4 WEEKS, AND THE PESTICIDE DOSES USED WERE 25 PPM (BLUE BARS), 50 PPM (GREEN BARS) OR 100 PPM (RED BARS). NUMBER OF TREATMENTS IS SHOWN ON TOP OF BARS.

Figure 7.14 shows that control of stable flies depends on the amount of larvicide used. The relationship between pesticide use (*CPD*) and control (*CI*) is approximately linear as long as *CPD* < 30,000 ml, but the linear relationship disappears when *CPD* exceeds 30,000 ml and reaches a maximum around 80%. This implies that strategies based on large amounts of pesticides do not necessarily lead to better fly control and will be a waste of pesticide and money. Such strategies are characterized by low values of *G/C*.

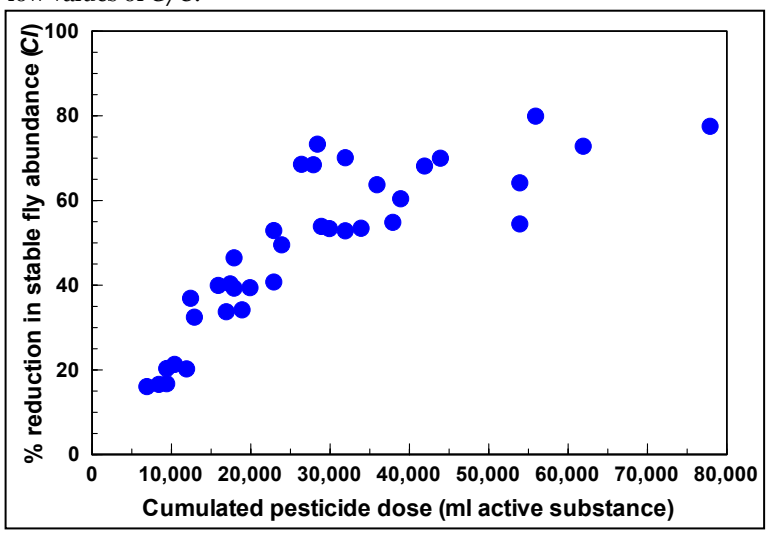

FIG. 7.14. RELATIONSHIP BETWEEN THE AMOUNT OF PESTICIDE USED AND THE PERCENT REDUCTION IN FLY ABUNDANCE (*CI*).

To understand why an increase in pesticide use may actually *increase FlyDays* compared with a lower dose, we run two simulations in which the dose was either 50 ppm or 100 ppm in the manure. Larvicide was applied adaptively using a treatment threshold of 10,000 adult individuals with at least 14 days between two treatments. Figure 7.15 shows that the low dose has a relative stabilizing effect on the fly population whereas the high dose destabilizes the fly numbers which fluctuate violently. As a consequence, control success of the former is 68.3%, but only 54.3% in the latter. In addition, *CPD* of the former is much lower (28,000 ml) than of the latter (42,000 ml). This means that the *G/C* value for the low dose strategy is 746.3 but only 395.6 for the high dose strategy.

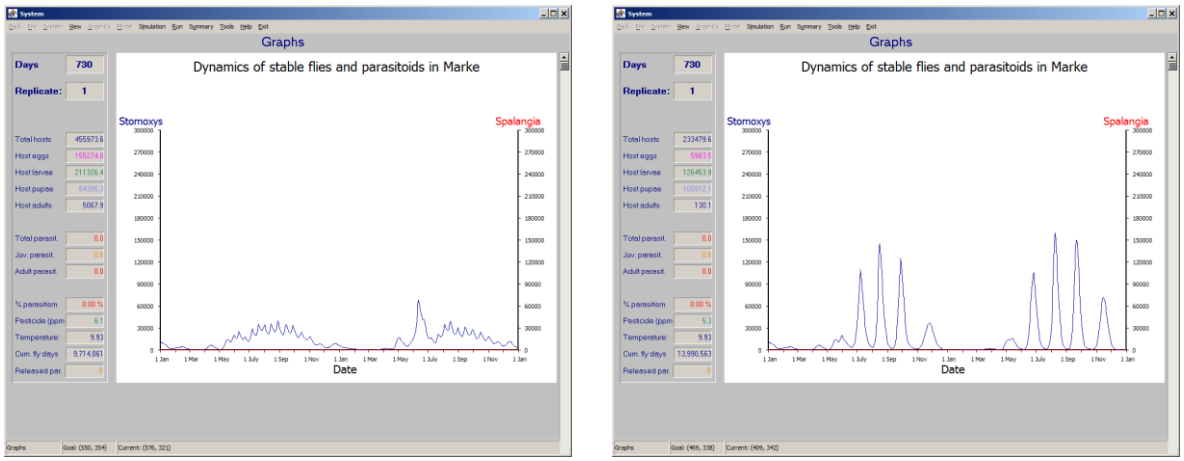

FIG. 7.15. THE DYNAMICS OF STABLE FLIES IF PESTICIDE IS APPLIED IN A DOSE CORRESPONDING TO EITHER 50 PPM (LEFT GRAPH) OR 100 PPM (RIGHT GRAPH). PESTICIDE IS APPLIED IF THE NUMBER OF ADULT FLIES EXCEEDS 10,000 INDIVIDUALS AND IF THE TIME SINCE THE LAST TREATMENT IS AT LEAST 2 WEEKS.

Figure 7.16 shows the per cent reduction in fly abundance (*CI*) when the dose per treatment is increased from 10 to 300 ppm larvicide. Treatment occurs every time fly abundance exceeds 10,000 adults, but separated by intervals of at least 14 days. *CPD* depends on the number of treatments and the dose applied per treatment. The figure shows that *CI* reaches a local maximum for *CPD* = 28,000 ml (corresponds to Fig. 7.16 left graph) and then declines to reach a local minimum at 42,000 ml (corresponds to Fig. 7.16 right graph). *CI* then starts to increase slowly but even when *CPD* exceeds 100,000 ml, *CI* is still below 80%. The *G/C* ratio peaks already at a *CPD* of 16,000 ml, though it is still high when *CPD* increases to 28,000 ml, but then declines steeply as more larvicide is applied.

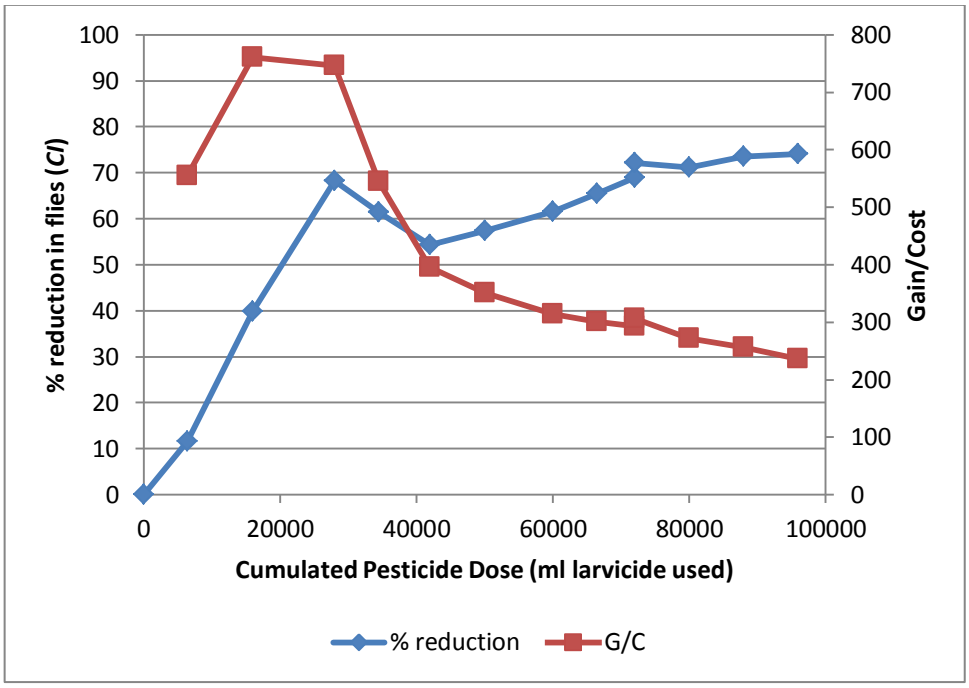

FIG. 7.16. THE PER CENT REDUCTION IN FLY ABUNDANCE (*CI*) AND THE GAIN/COST RATIO DEPICTED AGAINST THE AMOUNT OF PESTICIDES (*CPD*) USING AN ADAPTIVE STRATEGY BASED ON APPLYING LARVACIDE EACH TIME THE FLY ABUNDANCE EXCEEDS 10,000 ADULTS WITH AT LEAST 14 DAYS BETWEEN TREATMENTS. *CPD* DEPENDS ON THE NUMBER OF TREATMENTS AND THE DOSE PER TREATMENT.

7.4. Physical control by manure removal

In the previous simulations it was assumed that the depth of manure was 20 cm and that old manure was removed from the stable at the same pace as the cattle produced fresh manure so the net rate of change was zero. However, removal of manure usually takes place at irregular intervals where all or most manure in a compartment is removed at the same day. Depending on the number and size of the confined cattle, the manure will gradually accumulate again until the next cleansing. Removal of manure will cause substantial loss of the immature stages whereas the adult insects may escape by flying away.

We will use the model to investigate the influence of removing manure. The modelled stable consists of two separate compartments with different numbers of animals. A compartment is cleaned when the depth of manure in it exceeds a maximum acceptable value. After cleaning there is usually a small amount of manure left impeding total eradication of the immature stages. Thus, fresh manure is colonized by larvae originating from the left-overs as well as by adult flies.

Series 10 aims at investigating the consequences for the stable fly population when manure is removed each time its average depth exceeds a threshold depth. Three thresholds are used: 20, 30 or 40 cm. Each compartment is 50 m² and it is assumed that the daily production of new manure is 50 kg in compartment 1 and 60 kg in compartment 2. The simulations are started with 5000 kg manure in each compartment, corresponding to an average depth of 10 cm. After each cleaning, the average depth of the remaining manure is either 1 cm or 5 cm.

Figure 7.17 shows a run where manure is removed from a compartment each time its depth exceeds 30 cm.

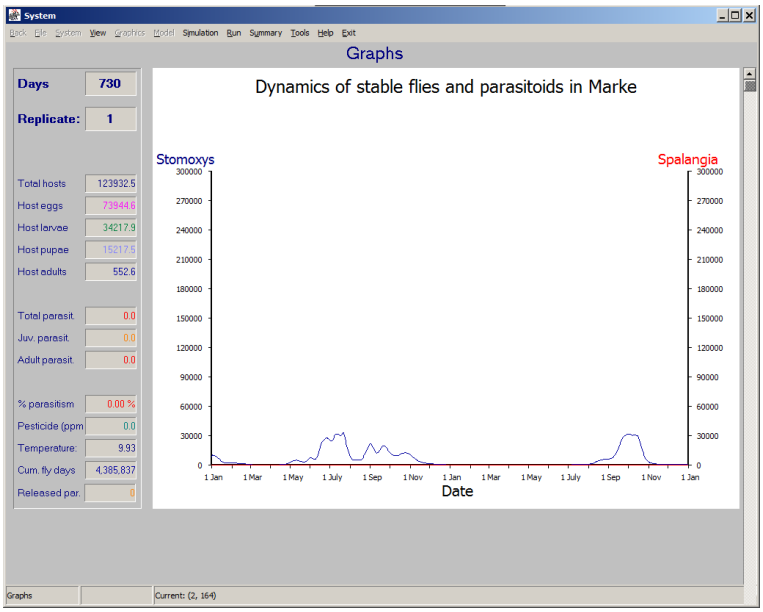

FIG. 7.17. A SAMPLE RUN FROM SERIES 10. MANURE IS REMOVED EACH TIME ITS DEPTH IN A COMPARTMENT EXCEEDS 30 CM. THE AVERAGE DEPTH OF MANURE IN A CLEANED COMPARTMENT IS 1 CM.

Figure 7.18 clearly shows that keeping a high sanitation standard in a stable can significantly reduce the abundance of stable flies. Careful cleaning, so that only 1 cm manure remains, requires fewer cleanings and gives lower abundances of flies than a more sloppy cleaning does. The number of cleanings can be reduced by postponing cleaning until the layer of manure exceeds, e.g., 30 or 40 cm compared with, e.g., 20 cm, but this will result in more flies. Consequently, the advantages of cleaning should be weighed against the costs associated with removal of manure.

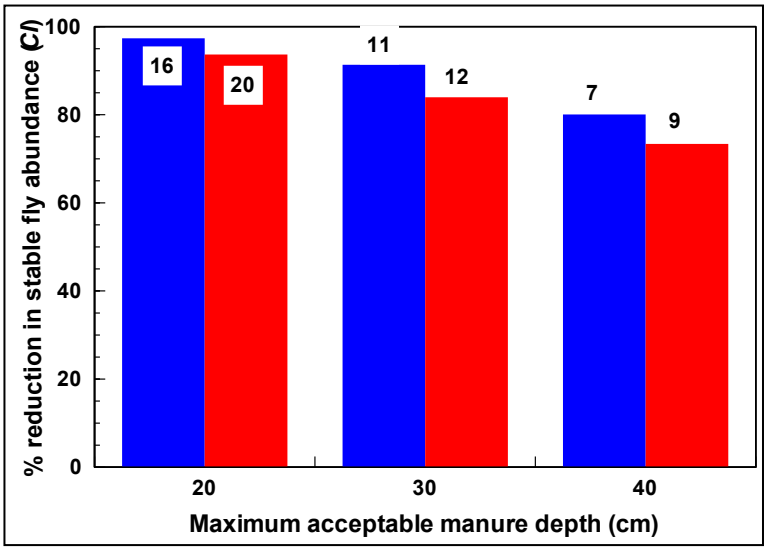

FIG. 7.18. EFFECT OF REMOVAL OF MANURE ON THE ABUNDANCE OF ADULT STABLE FLIES USING THREE DIFFERENT TREATMENT THRESHOLDS (20, 30, 40 CM). THE LEFT-OVERS AFTER A CLEANING ARE EITHER 1 CM (BLUE BAR) OR 5 CM (RED BAR).

7.5. Integrated control

The model allows for combining biological control with chemical and physical control strategies. However, as shown above a strategy based on a high sanitation standard renders the other control options superfluous. We therefore assume that the farmer applies a sanitation strategy based on continuous removal of manure so that the amount of manure in the stable remains constant and covers the floor in a 20 cm deep layer. In conventional livestock production, where pesticide applications until now have been the only way to get rid of flies, biological control in combination with chemical control might be an alternative. The integrated strategy is based on the philosophy that chemical control is applied as a supplement to biological control in periods where the natural enemies are insufficient to obtain control alone.

We will explore integrated control strategies where pre-planned releases of *S. cameroni* are combined with *ad hoc* applications of larvicide each time the abundance of stable flies exceeds a certain threshold. A pre-planned release strategy is optimal with respect to logistics whereas insecticides can be stored locally and used when needed. We assumed that the larvicide has no direct effect on *S. cameroni* as the immature stages develop inside puparia of *S. calcitrans*, but there may be an indirect effect on the parasitoids due to the lack of available hosts after a pesticide treatment.

Series 11 aims at testing three different thresholds for applying pesticide, namely when the number of adult flies exceeds 10,000, 20,000 and 40,000 individuals. The release strategy is based on 30,000 parasitoids every third week during the period from April 15 through October 15. Pesticide is applied in an amount corresponding to 25 ppm and the interval between two successive applications is at least 2 weeks. The integrated control strategies are compared with the outcome of the corresponding biological and chemical control strategies when they are used alone.

Series 12 is identical to Series 11 except that the amount of pesticide used per treatment is doubled so it corresponds to a dose of 50 ppm.

Series 13 is identical to Series 11 and 12 except that the amount of pesticide used per treatment corresponds to 100 ppm.

Figures 7.19-7.21 show that integrated control is always better than chemical control alone. When chemical control is used as part of an integrated control program, applications will only be needed at the low intervention thresholds, i.e. when the number of stable flies exceeds 10,000 or 20,000 individuals. When these two thresholds are used, integrated control is superior to the two other strategies used alone. However, the differences between integrated and chemical control are generally larger than the differences between integrated and biological control. The best result obtained with integrated control (85.5%) is achieved when high doses (100 ppm) of applications are used, but this will be at the cost of an extensive use of pesticide (*CPD* = 34,000 ml). In comparison, using low pesticide doses (25 ppm), a slightly lower control index is obtained (78.8%) but with a much lower *CPD* (14,000 ml). It may also be seen that using chemical control alone in doses of 50 ppm actually gives better control than doses of 100 ppm when the lowest intervention threshold is used and equal control at the intermediate intervention threshold.

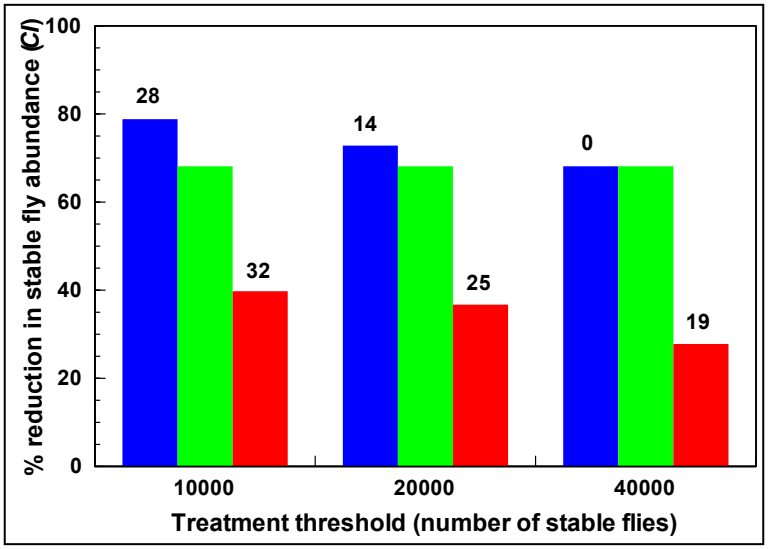

FIG. 7.19. RESULTS OF SERIES 11. THREE DIFFERENT STRATEGIES ARE COMPARED: INTEGRATED CONTROL (BLUE BARS), BIOLOGICAL CONTROL ALONE (GREEN BARS) AND CHEMICAL CONTROL ALONE (RED BARS). BIOLOGICAL CONTROLIS BASED ON PRE-PLANNED RELEASES OF 30,000 PARASITOIDS EVERY THIRD WEEK FROM APRIL 15 THROUGH OCTOBER 15, WHILE CHEMICAL CONTROL IS BASED ON PESTICIDE APPLICATION IN A DOSE OF 25 PPM WHEN THE ABUNDANCE OF STABLE FLIES EXCEEDS 10,000, 20,000 OR 40,000 INDIVIDUALS. THE NUMBERS OF PESTICIDE APPLICATIONS USED FOR INTEGRATED AND CHEMICAL CONTROL ARE SHOWN ON TOP OF THE BARS.

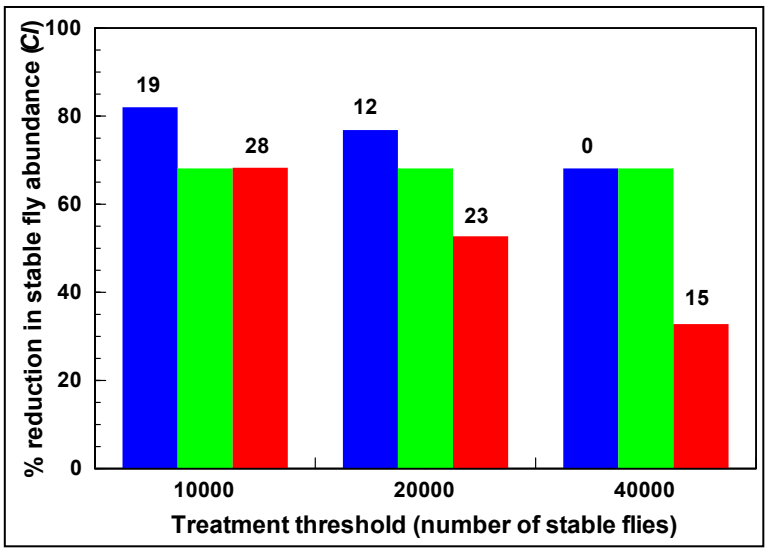

FIG. 7.20. RESULTS OF SERIES 12. THREE DIFFERENT STRATEGIES ARE COMPARED: INTEGRATED CONTROL (BLUE BARS), BIOLOGICAL CONTROL ALONE (GREEN BARS) AND CHEMICAL CONTROL ALONE (RED BARS). BIOLOGICAL CONTROLIS BASED ON PRE-PLANNED RELEASES OF 30,000 PARASITOIDS EVERY THIRD WEEK FROM APRIL 15 THROUGH OCTOBER 15, WHILE CHEMICAL CONTROL IS BASED ON PESTICIDE APPLICATION IN A DOSE OF 50 PPM WHEN THE ABUNDANCE OF STABLE FLIES EXCEEDS 10,000, 20,000 OR 40,000 INDIVIDUALS. THE NUMBERS OF PESTICIDE APPLICATIONS USED FOR INTEGRATED AND CHEMICAL CONTROL ARE SHOWN ON TOP OF THE BARS.

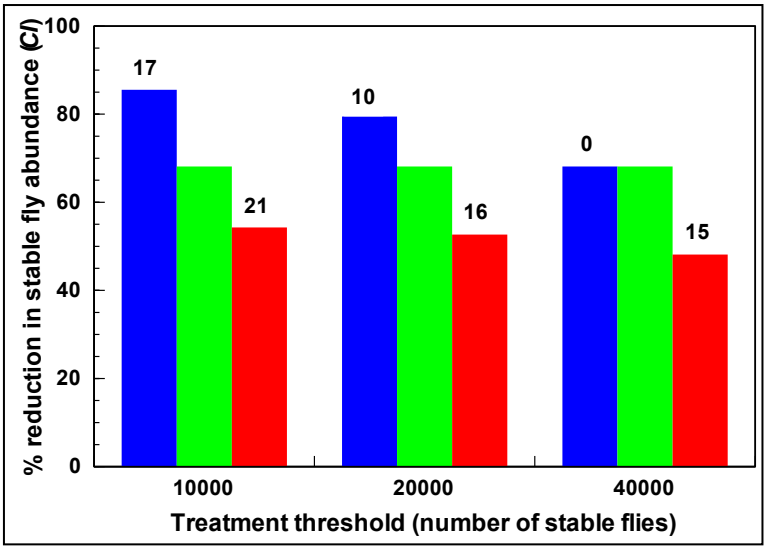

FIG. 7.21. RESULTS OF SERIES 12. THREE DIFFERENT STRATEGIES ARE COMPARED: INTEGRATED CONTROL (BLUE BARS), BIOLOGICAL CONTROL ALONE (GREEN BARS) AND CHEMICAL CONTROL ALONE (RED BARS). BIOLOGICAL CONTROL IS BASED ON PRE-PLANNED RELEASES OF 30,000 PARASITOIDS EVERY THIRD WEEK FROM APRIL 15 THROUGH OCTOBER 15, WHILE CHEMICAL CONTROL IS BASED ON PESTICIDE APPLICATION IN A DOSE OF 100 PPM WHEN THE ABUNDANCE OF STABLE FLIES EXCEEDS 10,000, 20,000 OR 40,000 INDIVIDUALS. THE NUMBERS OF PESTICIDE APPLICATIONS USED FOR INTEGRATED AND CHEMICAL CONTROL ARE SHOWN ON TOP OF THE BARS.

8. Discussion

8.1. The model

In this report we have presented a fairly general mathematical model of a parasitoid-host system. Because the model is not data-driven but based on general principles derived from parasitoid-host theory (see e.g. Briggs et al. 1999), the model may apply to a variety of parasitoid-host systems. However, as our aim was develop a model that could be used to analyse a specific system consisting of stable flies and the pupal parasitoid *S. cameroni*, we used these two species to parameterize the model. Consequently, the realism of the model has to be evaluated on basis of how well its assumptions and predictions match with data obtained from laboratory and field experiments with the stable fly *and S. cameroni.* Finally, based on the mathematical model and the experimental data, we developed a simulation program called *The Fly Simulator*, which serves as an interface between the user and the underlying mathematical model. The program can be used without prior knowledge of the underlying mathematics. This makes the model user-friendly, but at the expense of transparency. We will therefore present some of the assumptions and simplifications we have made during model development and discuss the relevance of these attempts in relation to the specific stable system. A model is always a simplification of the real system, so it is necessary to make a compromise between simplicity and realism. By presenting our premises for reaching such compromises, we hope that the readers will be able to give us some feed-back and suggestions as to how the model's realism can be increased without increasing its complexity significantly.

8.1.1. The structure of the modelled populations

As the biology and ecology of the different life-stages in an insect's life-cycle (eggs, larvae, pupae and adults) are very different, we decided to apply a stage-structured modelling approach (Briggs et al. 1999). We further included an age-structure within each developmental stage in order to keep track of all individuals entering the stage at the same day (a cohort). We ignored individual variation among individuals within the same age class. Otherwise we should have used an individual-based approach which would have prolonged simulation time enormously and required very powerful computers (Grimm and Railsback 2005). Moreover, in order to parameterize an individual-based model we would have needed data obtained at the individual level. The populations were subdivided into males and females, but for simplicity we assumed that the two genders are identical with respect to developmental and survival rates. We also assumed that female fecundity is independent of male abundance.

8.1.2. The limits to the modelled system

We decided to consider the stable as a closed system with respect to immigration and emigration of insects although adult stable flies are capable of moving over considerable distances (Hogsette et al. 1989). Thus, according to the model eradication of stable flies is possible, whereas in reality the stable can be recolonized by immigrants arriving from outside the stable or the farm. Though the model allows for immigration of adult stable flies, we have for simplicity set the number of immigrants per day to zero, partly because we consider the contribution of immigrants to the total population of stable flies as negligible and partly because we do not have sufficient data to model migration realistically.

8.1.3. External factors

In contrast to most natural ecosystems, where temperature, precipitation, relative humidity, wind speed and solar radiation can affect the system as driving variables, we considered only temperature to be of importance because relative humidity inside a stable is usually high and probably never a limiting factor. Temperature is known to be a very important predictor for insect development, survival and fecundity (e.g. Mironidis & Savopoulou-Soultani 2008). We assumed that

the biological processes taking place in the stable are influenced by the air temperature inside the stable which in turn is affected by the weather outside the stable. Preferably air temperature should be measured directly but if temperature data are missing, they can be estimated from outdoor temperatures obtained from the nearest meteorological station as demonstrated in Section A2.4.

8.1.4. Manure quality

Flies and parasitoids spend their immature stages in a substrate consisting of a mixture of straw, faeces, urine, and more or less decomposed feed. Although this is a very heterogeneous mixture with a significant vertical component, we chose to ignore this fact and simply regarded the substrate as a black box (called manure). The amount of manure is assumed to be either constant, reflecting a balance between production and removal, or to change with time depending on how frequently the stable is cleaned.

8.1.5. Manure temperature

In an early version of the model we assumed that the temperature in the manure was constant both vertically and temporally, but it soon became evident that temperature variations in the manure are important as a driver for the system. Thus, the low manure temperatures during winter time slow down development of fly larvae and thereby enabling this stage, as the only one, to survive throughout the entire winter to become pupae when temperatures start to rise again in spring. Fermentation of organic material tends to increase temperature in the manure relative to the air temperature, which protects the immature stages against extremely low temperatures during winter time. On the other hand, manure buffers against extremely high air temperatures during summer time. We therefore incorporated a fairly simple submodel based on the ambient temperatures (air and floor temperature), heat production due to fermentation and heat transfer due to conduction. The model considerably improved the realism of the model.

8.1.6. Spatial variation

Conditions for the insects may vary between the different parts of the stable. At an early stage in the model development, we decided that an individual-based model, where the position of each individual in a population is modelled explicitly, was not relevant of reasons given previously. Instead we applied a population-based modelling approach where all individuals of a given stage staying in a given area are assumed to experience the same environment. We divided the stable into smaller areas occupied by cattle (called compartments) and allowed the populations to develop independently in each compartment, though they share the same ambient temperatures. Only adult insects could move from one compartment to another. The current version of *The Fly Simulator* divides the stable into two separate compartments (as in Marke), but later versions of the program can be extended to include more compartments.

8.1.7. Density-dependent factors

All natural populations are affected by negative density-dependent factors, i.e., mortality factors that increase the per capita mortality rate as population density increases, which will act as a negative feed-back mechanism on population growth. We assumed that the stable flies are limited by scramble competition among larvae in the manure (see e.g. Nicholson 1954), leading to high per capita mortality when larval density is high. We assumed that the only limiting factor is the quantity of manure although high larval density may also affect the composition of the manure qualitatively. We assumed that adult stable flies have unlimited access to food (blood) and do not compete for suitable sites to lay eggs (Shofield & Torr 2002). Parasitism of fly pupae is positively density-dependent because the functional response of *S. cameroni* is modelled as a type II response. A positive density-dependent factor *per se* will not be able to stabilize the density of stable flies because the mortality risk per pupae declines with fly density.

The parasitoid females are assumed to search for hosts at random so encounters with healthy and already parasitized pupae occur in proportion to the abundance of these two groups of hosts. The assumption of random search is based on the principle of Occam's razor because we have no data indicating that non-random search is more realistic. The density of parasitoids is limited by the number of healthy fly pupae and the intra-specific competition among the adult female

parasitoids for finding suitable hosts (mutual interference). When the ratio between female parasitoids and fly pupae is high, it becomes more likely that two or more eggs are placed in the same puparium, a phenomenon known as superparasitism. In the model it is assumed that only the first egg survives to adulthood, although it happens that none of the offspring survives due to the intense competition among the parasitoid larvae (Skovgård & Nachman 2015b).

8.1.8. Deterministic versus stochastic modelling

In contrast to a deterministic model, which predicts the expected behaviour of the system for a given initial state and a given set of temperature data, a stochastic model describes a unique realization of the system. Thus, a stochastic model can be used to find the probability that a given event, or one which is more extreme, will happen, for instance the likelihood that stable flies will exceed a certain threshold density when some control measures have been taken. In small populations, demographic stochasticity associated with births, deaths, dispersal etc. may have a profound effect on population dynamics (see e.g. Nisbet & Gurney 1982). However, the role played by demographic stochasticity declines with population size, typically in populations exceeding more than 50-100 individuals. Therefore, we do not see any need of incorporating demographic stochasticity which otherwise would make the model more complicated, increase computation time and require replicated runs to generalize its output. Furthermore, as we used real data to simulate variation in temperature, there was no need of including environmental stochasticity. However, environmental stochasticity was incorporated in the sensitivity analysis to investigate the system's sensitivity to variation in ambient temperature.

8.1.9. Intervention options

Although the primary purpose of the model is to apply it for investigating the feasibility of using *S. cameroni* as biological control agent against stable flies, we also included two other control options in the model, namely chemical control by means of a larvicide and physical control by means of manure removal. The model assumes that the pesticide is homogeneously distributed in the manure, although it is likely that the concentration is higher in the top layer and then declines downwards. It is further assumed that the likelihood that a larva dies due to larvicide is independent of larval density although it seems likely that starving larvae are more sensitive to pesticide than well-nourished. Finally, we assume that the lethality and the degradation of the pesticide are independent of temperature. We acknowledge that these assumptions are very simplistic and should be scrutinized in case the model should be used for more realistic analyses of chemical control. Likewise, the effect of manure removal is also very simplistic and based on the principle of Occam's razor, i.e. we assume that the proportion of removed insects (immatures of both species and adult parasitoids) is the same as the proportion of manure removed, whereas it does not affect the mortality of adult flies. This is probably a reasonable assumption with respect to the stable flies but not *S. cameroni*, because adult parasitoids may also spend time outside the manure.

With respect to biological control, we assume that inundative releases are based on 0 days old parasitoids, although in practice the parasitoids are likely to differ with respect to age (Skovgård 2004). It is also assumed that the sex ratio of the released parasitoids is the same as of the offspring produced by the resident parasitoid population. In practice, however, we cannot expect that a mass-production system is capable of standardizing parasitoid production to an extent that considerable variations in age and sex distributions can be avoided.

8.2. The data

We used empirical data originating from many different sources to parameterize the model with values specific for the *S. calcitrans*-*S. cameroni* system. Although the data have been obtained under different experimental conditions and may vary considerably with respect to quality, they nevertheless represent the best achievable at the time being. As more or better data become available they may supplement or replace the existing ones so as to increase the precision of the parameter values and thereby improve the quantitative predictions of the model. New data may also be used to revise the model's functional relationships as some of these are based on provisional data. In the latter case, it may lead to structural changes of the model, i.e., by changing its equations or by adding new functional relationships.

Whereas we have quite substantial data sets obtained from laboratory experiments, we lack data obtained under field (i.e. stable) conditions allowing us to test the predictions of the model. In fact, we only have one good data set of stable fly dynamics, namely the monthly population estimates obtained by means of mark-recapture samplings in Marke (Skovgård & Nachman 2012). This data set was used both for calibration of the parameter expressing competition among fly larvae and for validating the model. We therefore lack independent data sets to be used for a proper validation of the model. Ideally, new data sets should be acquired by means of mark-recapture sampling as this method is likely to give the most accurate estimates of the entire population size (Nachman & Skovgård 2012). Unfortunately, mark-recapture is a very laborious procedure to apply so instead index values are often used as a quick, but imprecise, short-cut to assess relative changes in population densities (see Section 2.1.4). For instance, Skovgård (2004) used the average number of flies per animal based on the method of Kristiansen & Skovmand (1985) to compare fly development on four Danish organic farms (two with and two without inundative releases of *S. cameroni*), while Skovgård & Nachman (2004) used the same method to follow the dynamics of flies on three farms over two consecutive years. As the farms were only treated with *S. cameroni* during the second year, the significantly lower fly abundances in this year compared with the preceding year was attributed to the effect of parasitoids.

8.2.1. Modelling stable flies

Larsen & Thomsen (1941) and Sutherland (1979) provide data on average developmental time and total viability of *S. calcitrans* eggs, larvae and pupae stable flies (Figs. A2.1-A2.6 in Appendix 2). For each stage, we used these data to estimate the daily development, survival and hatch rates by assuming that hatch rate is age-dependent while daily development and survival rates are age-independent. Survival rate is assumed to be directly influenced by the ambient temperature, whereas the hatch rate is modelled by means of biological age (Q) which is an increasing function of the cumulated temperature above a lower threshold. Thus, the biological age of an individual depends on the temperature regime it has experienced so that development is faster (in chronological time), the higher the ambient temperatures.

We applied a generic model (called SANDY) to model daily development, survival and hatching of each stage separately, using an Excel spreadsheet where the state variables were updated on a daily basis to simulate the fate of a cohort. The parameters of the SANDY model were estimated as the values that yield the best agreement with the data of Larsen & Thomsen (1941) and Sutherland (1979). The solutions were obtained by iteratively changing the parameters. This procedure may incur some risks because the lower and upper temperature thresholds for development (T_{min} and T_{max}) in most instances lie outside the range of empirical data and therefore subject to uncertainty. We further assumed that T_{min} and T_{max} for survival are the same as for development in order to limit the number of estimated parameters. However, it is likely that individuals can survive (at least for a short time) at temperatures outside the temperature range allowing for development. Especially eggs are likely to sustain prolonged periods of low temperatures, so for this stage we estimated two different values of T_{min}, one for development (T_{min} = 11.26°C) and one for survival (T_{min} =6.31°C). It means that we assume that no eggs will survive after 24 hours exposure to temperatures below 6.31°C. We have no data allowing us to test this prediction.

Adult survival and oviposition rates were also modelled by means of the SANDY model. We used data by Sutherland (1979) on MT_{50} (mean time until 50% of a cohort has died) and life-time egg production per female measured at different temperatures to estimate the parameters of the SANDY model (Figs. A2.7 and A2.8 in Appendix 2). Both survival and fecundity rates were found to be temperature-dependent. In contrast to the age-independent survival rates of the immatures, we assumed that the daily survival rates of adult flies declines with biological age. Daily oviposition rates also depend on the age of the females. It first increases steeply with Q to reach a maximum when the females are still young and then declines steadily towards 0. The effects of biological age on survival and oviposition rates were estimated from a data set provided by Salem et al. (2012) where only one temperature (25°C) was used (Fig. A2.9). Similar data sets obtained at a wider range of temperatures would be useful in order to test the generality of the analyses.

All stages of *S. calcitrans* were found to be very dependent on the ambient temperature. The larval stage is supposed to be the main stage of stable flies during winter time. As data indicate that the lower threshold for development and survival of larvae is (T_{min}) is ca 3°C, we expect that this is the lower limit for overwintering populations of stable flies. During summer time, larvae and pupae are predicted to die if the manure temperature exceeds ca 35° whereas the other stages are less sensitive to high temperatures. The adult flies are assumed to survive and reproduce if the ambient temperature

is above ca 10°C whereas we were unable to estimate the upper temperature threshold. We therefore set T_{max} to 45°C. The maximum oviposition rate per female (F_{max}) was estimated to be 11.86 eggs/day obtained at 21.7°C.

When the life-table data are combined into a full life-time cohort, it allows us to estimate the net reproductive rate (R_0) and the intrinsic rate of natural increase (r_m) at any given temperature. Both parameters assume that there is no intraspecific competition and food is unlimited (i.e. the competition parameter ψ is 0).The values of R_0 at 15, 20, 25, 30 and 35°C are estimated to be 0.000016, 12.36, 12.95, 5.04 and 0 per generation, and r_m at the same temperatures as <0, 0.041, 0.087, 0.071, <0 per day. The maximum value of R_0 (= 17.21 per generation) occurs at 21.9°C while the highest r_m (= 0.090 day^{-1}) occurs at 25.9°C. Duration of the immature stages is estimated to 62.0, 30.2, 19.2, and 14.7 days, and survival rate from egg to adult to 18.8%, 49.2%, 49.1%, and 31.1% at 15, 20, 25, and 30°C, respectively, while no individuals survive at 35°C. Mean generation time (G) at 15, 20, 25, and 30°C are 108.3, 64.8, 30.0, 23.0 days, respectively. The shortest doubling time is 7.70 days.

When the life-history parameters used in our study are compared with other studies, some discrepancies become obvious. The most important difference is found when life-time oviposition and daily fecundity rates are compared. We based our analysis on data provided by Sutherland (1979) who found a maximum life-time reproduction of about 60 eggs per female, which is much smaller than the value of about 750 given by Lysyk (1998). Consequently, the values of R_0 obtained by Lysyk are 12.2, 135.1, 143.0, 84.0, and 6.3 per generation at 15, 20, 25, 30 and 35°C, respectively. Mean generation times at the same temperatures were calculated as 92.0, 54.2, 32.5, 23.0, and 21.8 days, and r_m as 0.023, 0.110, 0.213, 0.24, and 0.103 day^{-1}.

Gilles et al. (2005a) give the survival probabilities from egg to adult at 15, 20, 25, 30 and 35°C as 50%, 84%, 83%, 74% and 35%, respectively, which are considerably higher than estimated from the combined data provided by Larsen & Thomsen (1941) and Sutherland (1979). The developmental times from egg to adult were found by Gilles et al. (2005a) as 70.7, 32.4, 16.7, 12.9 and 13.2 days at the same five temperatures, which agree quite well with the values used in our study. Furthermore, Gilles et al. (2005b) also calculated R_0 as 3.9, 26.3, 11.8, 6.6, and 0 per generation at 15, 20, 25, 30 and 35°C, mean generation time as 104.3, 62.8, 35.0 and 21.1 days, and r_m as 0.013, 0.052, 0.077, and 0.093 d^{-1} at 15, 20, 25 and 30°C (the values were read off from Fig. 3 in Gilles et al. (2005b) and are therefore approximate). Salem et al (2012) gives the values of R_0, r_m and mean generation time at 25°C as 16.2, 0.09 day^{-1} and 31 days, respectively, which agree quite well with the values found in the present study.

The low fecundity potential found by Sutherland (1979) might be due to crowding effects among ovipositing females as 150-200 adults were kept in the same cage, whereas Lysyk (1998) used only 50 adults of which half were females in his experiments. Another cause for the differences might be due to the quality of the food used to feed the ovipositing females. However, the values of R_0 and r_m presented by Gilles et al. (2005b) and Salem et al. (2012) are more in line with those used in our simulations than with those of Lysyk (1998), but we suspect that the simulation model underestimates the ability of stable flies to increase in numbers given the right opportunities.

Finally, Skovgård & Nachman (2004, 2012) estimated r from field data and found that the realized growth rate (r_m) was positive at temperatures above 10-12°C and peaked at about 22°C. Maximum per capita growth rate was estimated to be 0.03 d^{-1} (95% CL: 0.02-0.06 d^{-1}) based on fly abundance indices (Skovgård & Nachman 2004) and to be 0.070 d^{-1} (95% CL: 0.014-0.12 day^{-1}) based on mark-recapture data (Skovgård & Nachman 2012). The relatively low growth rates estimated from field data are likely due to a number of mortality factors not present in the laboratory experiments (e.g. unsuitable manure, interspecific competition with other fly species, predation etc). Consequently, the maximum value of r_m = 0.090 d^{-1} used in the simulation model seems to reflect field conditions more realistically than the very high growth rates found from laboratory experiments.

8.2.2. Modelling *Spalangia cameroni*

Like stable flies, *S. cameroni* responds strongly to temperature. The analysis based on Skovgård & Nachman (subm.) shows that the species can survive and reproduce at temperatures in the range between 15 and 35°C, but temperatures between 25 and 34°C seem to be the optimal range (Figs. A2.10-A2.13). Likewise, Moon et al. (1982) found that the daily survival rate was high in the range between 10°C and 28°C, but decreased to 0 at 37.5°C. The activity level of *S. cameroni* also depends strongly on temperature with a maximum around 28°C. Oviposition rate depends on the attack rate and success ratio, and as both peak around 28°C, oviposition has a maximum at 27.9°C where the daily rate of successful

parasitism (attacks resulting in a viable offspring) is found to be 18.2 offspring per female (Figs. A2.14-A2.16). Moon et al. (1982) estimated the daily reproduction to peak at 11 progeny/female at 31.7°C. The maximum attack and oviposition rates in Skovgård & Nachman (2015a) are higher than the values estimated from the experiments by Skovgård & Nachman (subm.). A likely explanation for the discrepancy between the two studies is that Skovgård & Nachman (2015a) used young females which were allowed to lay eggs for one day. They therefore had an opportunity to exploit their egg load and did not waste time on host feeding. In contrast, Skovgård & Nachman (subm.) followed the same females over several days, implying that the females had to supplement energy for egg production by host feeding (Quicke 1997). It should also be noticed that Skovgård & Nachman (subm.) used house flies as the host while Skovgård & Nachman (2015a) used stable flies. Despite host feeding may contribute to reducing attack and oviposition rates in a stable, we believe that host density usually is so low that attacks and ovipositions are constrained by access to fly pupae rather than by lack of fertile eggs. We therefore used the data from Skovgård & Nachman (2015a) to estimate the parameters associated with attack and oviposition rates, and the data from Skovgård & Nachman (subm.) to estimate the parameters associated with survival rates.

We included mutual interference among females of *S. cameroni* in the simulation model although data by Skovgård & Nachman (2015b) indicate that the mean density of adult parasitoids in a stable usually will be far below the densities where mutual interference reduces the attack rate significantly. Thus, the density should exceed ca 0.5 females per cm^2, which corresponds to 5000 females per m^2 (Figs. A2.17-A2.19). However, shortly after releases, the density of parasitoids may locally be so high that mutually interference might reduce their efficiency. It means that better control cannot be achieved by just increasing the number of released parasitoids as this will merely exaggerate the problem. This phenomenon has been observed when whiteflies are controlled by means of inundative releases of *Encarsia formosa* (Hoddle et al. 1997).

Data by Larsen (2006) and Moon et al. (1982) indicate that the parasitoids have preference for attacking pupae of intermediate ages (expressed in terms of biological age). If they have no choice, they are capable of attacking and ovipositing on both young and old pupae (Figs. A2.20-A2.21), but if they are given a chance to choose, pupae of intermediate age are used for ovipositing while the other age groups are used for host feeding (Figs. A2.22-A2.23). We fitted preference functions to the data, but as the data set provided by Larsen (2006) is rather small, these functions need validation against other data sets in order to test their generality.

When the life-table data of Skovgård & Nachman (subm.) are combined into a full life-time cohort for *S. cameroni*, the values of R_0 at 15, 20, 25, 30 and 35°C are estimated to be 0, 28.95, 33.26, 33.04 and 0 per generation, and r_m at the same temperatures as <0, 0.060, 0.103, 0.134, 0.142, <0 per day. The maximum value of R_0 (= 33.44 per generation) occurs at 26.2°C while the highest r_m (= 0.153 day^{-1}) occurs at 32.8°C. Duration at the immature stages is estimated to 113.2, 45.3, 27.0, and 19.4 and 34.4 days, and survival rate from egg to adult to 51.7%, 73.7%, 77.9%, 75.5% and 3.3% at 15, 20, 25, 30 and 35°C, respectively. Mean generation time (G) at 20, 25, and 30°C is estimated to be 58.9, 36.2, and 25.9 days, respectively. The shortest doubling time is 4.22 days, while Moon et al (1982) give the maximum value of R_0 as 19.8.

The estimated values of R_0 and r_m for most temperatures are very high, especially when they are compared with those of the stable fly. However, it has to be noticed that R_0 and r_m assume that there are no limiting factors, allowing the female parasitoids to realize their maximum reproductive capacity. This will require very high densities of host pupae, so in practice the potential growth rates are not likely to be realized under natural conditions. However, the high reproductive potential of *S. cameroni* may be an advantage when the species is mass produced.

8.2.3. Modelling pesticide application

In order to include pesticide induced mortality in the model, we used data by Miller & Chamberlain (1989). They give information about the relationship between dose and lethality of the larvicide azadirachtin (Fig. A2.24). As we have no information on how the pesticide decays in manure, we assumed that its concentration in the manure declines with 10% per day (corresponding to a half-time of 6.93 days). We recognize that better data are needed in case the simulation model should be applied for analysing the effect of a specific pesticide.

8.2.4. Modelling environmental factors

We used outside temperatures measured by nearby meteorological stations to estimate stable temperatures during periods where such temperatures were not measured directly. For simplicity the relationship between outside and inside air temperatures was assumed to be linear (Figs A2.25 and A2.26). Although this assumption is reasonably met at the two farms from which data are available, Fig. A2.25 indicates that temperatures inside the stable tend to be underestimated when outside temperatures are either very low or very high, implying that a curvi-linear relationship would provide a slightly better fit to data.

8.2.5. Modelling larval competition

Unfortunately, we lack experimental data suitable for analysing intraspecific competition among stable fly larvae. Many studies have demonstrated that larvae of diptera compete for resources (e.g. for food and space) and both exploitation and interference competition can be involved (Nicholson 1954, Mueller & Joshi 2000). Exploitation competition occurs when a resource removed by one individual is no longer available for other individuals, while interference competition is characterized by direct antagonistic behaviour between conspecifics (Smith & Smith 2009). We assumed that all larvae are identical with respect to competitive abilities so the effect of increasing larval density is equally distributed among all larvae in the population, irrespective of their age. This leads to scramble competition (Smith & Smith 2009). Scramble competition affects population growth negatively when population density increases. Such negative feed-back mechanisms can either be compensating, leading to damped oscillations, or be over-compensating which, in combination with time-lags, can lead to violent oscillations, typically occurring in species with a high reproductive potential (May 1974).

By calibrating the competition parameter (ψ) to the observed population dynamics of stable flies we found that $\psi = 20$ cm^3/larvae gave an acceptable fit of the model to the sampling data. The analysis of the model (Section 5.2) shows that the expected effect of intraspecific competition is to dampen fluctuations (Fig. 5.1), so that the population size will approach an equilibrium which depends on temperature (Fig. 5.2).

Since ψ was not estimated from independent data, the current value of ψ depends on the values of all the remaining parameters of the model. Thus, if one or more of these parameter values are changed, ψ needs to be recalibrated. Furthermore, as the value of ψ was obtained from a single stable, we do not know whether this value also applies to other stables. Consequently, it would a big advantage if an experimentally determined value of ψ could be used instead. Experiments where different numbers of eggs or young larvae are added to a fixed amount of substrate and where the subsequent numbers of pupae or adults are counted could provide experimental evidence of how crowding affects survival rates of stable fly larvae. Finally, it should be noted that ψ when estimated from field data, although termed the larval competition coefficient, is likely to encompass the combined effect of all density-dependent factors acting simultaneously on the larval stage, e.g., competition with larvae of other fly species, diseases, predation by beetles and mites (Willhoit et al. 1991). Nicholson found that survival of blowfly larvae declines steeply with increasing larval density (see Fig. 4.1 in Mueller & Joshi 2000). Besides, intraspecific competition among fly larvae may also prolong their development time and diminish the size at adulthood, which in turn may leads to reduced fecundity (Mueller & Joshi 2000).

The model explains 64% of the variation in sample data when ψ was fitted to data. The explained variation could be increased further by calibrating the initial stage distribution, that is, if we drop the assumption that the initial and final stage distributions should be identical and instead vary the initial distribution until the maximum explained variation is achieved. Although it is obvious that it is highly unlikely that the initial and final stage distributions are identical, it is, however, the simplest assumption to make according to principle of Occam's razor. Changing the initial stage distribution just in order to obtain a better fit becomes nothing else than a curve-fitting exercise because the explained variation of a model is expected to increase, the more variables that are allowed to change. With only 22 data points available, we found that it would be most appropriate to limit the number of estimated parameters to a single one, implying that the residual variance has 21 degrees of freedom.

8.3. The analyses of the system's fluctuations and stability

The purpose of the analyses was to understand the internal dynamics generated by the model in a constant environment, that is, to investigate what kind of dynamics its functional relationships can produce given the model's parameters. The analyses focused primarily on using the parameter values relevant for the *S. calcitrans-S. cameroni* system, whereas a full analysis should investigate a wider range of parameter values. However, by examining the system's properties at different, but constant, temperatures, we examined a rather broad spectrum of temperature-dependent parameters affecting development, survival and oviposition rates. Only in case of stable fly fecundity we extended the parameter space outside the one obtained experimentally by increasing maximum oviposition rate (F_{max}) from 11.86 to 16 eggs produced per female per day in order to examine whether this could change the dynamics of stable flies from damped oscillations to stable limit cycles in absence of parasitoids.

Using the default parameters values showed that larval competition is capable of stabilizing the density of stable flies so that population density in absence of environmental and demographic stochasticity will approach an equilibrium, which depends on temperature (Figs. 5.1 and 5.2). However, as shown in Fig. 5.3, increasing fecundity with about one-third (e.g. to 16 eggs/female/day) can change the dynamics to become cyclic with a constant period and amplitude (stable limit cycles) at intermediate temperatures where the realized fecundity is highest. Hence, the model can generate dynamics similar to that exhibited by blowflies in the experiments conducted by Nicholson (1954). Mueller & Joshi (2000) reviewed theories concerning cyclic dynamics in blowflies and attributed the cycles to high baseline fecundity in combination with a strong time-delayed adult density-dependent recruitment. May (1974) generalized this finding by stating that a population's dynamics may switch from a stable equilibrium point to a stable limit cycle as "life gets better".

When parasitoids were added to the system, both hosts and parasitoids exhibited stable limit cycles at optimal temperatures and equilibrium dynamics (after a transition period with damped oscillations) at sub-optimal conditions (Figs. 5.4 and 5.5). This is also in agreement with May (1974) who suggested that prey-predator (host-parasitoid) systems will exhibit stable limit cycles when there is relatively weak intraspecific competition among prey and the intrinsic growth rate of the prey population exceeds that of it predators.

The simulations indicate that the optimal temperature for keeping a culture of stable flies under laboratory conditions is around 28°C as its productivity is highest at this temperature. The same applies to the productivity of *S. cameroni*, but the model predicts that a culture *S. cameroni* maintained on stable flies will exhibit violent fluctuations. The largest amplitude of the cycles occurs at 28°C where stable flies vary between 9,363 and 2,697,133 individuals and the parasitoids vary between 605 and 294,639 individuals. Paradoxically, the number of stable flies in presence of parasitoids can exceed the number when parasitoids are absent (2,323,669 individuals).

Violent population cycles increase the risk that one or both species go extinct during periods of low density. This may have some ramifications for keeping flies and parasitoids in laboratory cultures. Thus, if the model is down-scaled from a system consisting of 100 m² to a small laboratory system of, e. g., 0.25 m², the minimum population sizes at 28°C are expected to be 23.4 stable flies and 1.5 parasitoids. Such low population sizes will make the populations, and especially the parasitoids, very vulnerable to demographic stochasticity and may lead to extinction even if the environment is constant (i.e. no environmental stochasticity) (see e.g. Nisbet & Gurney 1982).

8.4. Sensitivity analysis

The sensitivity analysis aims at revealing which of the many parameters in the parasitoid-host model that are most sensitivity to uncertainty. This may help to identify where more precise data will be useful to improve reliability, for instance by conducting specific experiments. As the model contains 97 parameters, a comprehensive sensitivity analysis of all parameters and their interactions would be completely unmanageable. Instead, we conducted a screening procedure by subjecting each parameter to only two values, one smaller and one larger than the standard value, in order to investigate the effect on the response variable (in casu *FlyDays*). Moreover, we only used one sequence of random temperature fluctuations. Although this may suffice to give an approximate ranking of parameters with respect to sensitivity, it does not take variability in elasticity into consideration as demonstrated when 10 different values of e for ψ and L_2 were generated by applying different sequences of temperature fluctuations. Although the average e was quite close to the single point estimate of e for both parameters, this outcome is attributed to pure coincidence, as the variability in the individual values was considerable.

The analysis pointed at 14 sensitive parameters of which 12 have been estimated from independent experimental data and can therefore be regarded as reasonable reliable. The remaining two parameters are ψ (expressing intraspecific competition among stable fly larvae) and L_2 (the maximum depth in the manure where fly pupae can be found and parasitized by adult parasitoid females). The former is obtained by means of calibration, while the latter is a guesstimate. Thus, experiments that can yield reliable estimates of ψ and L_2 would be very beneficial for validating the model.

Overall, the most sensitive parameters are associated with survival of fly larvae and pupae, and of the immature stage of the parasitoid (Figs. 6.1-6.3). Even a small change in s_{max} can lead to substantial changes in population dynamics and, as a consequence, in *FlyDays*. It is, however, not surprising as the likelihood that an individual survives n days with a daily survival rate of s becomes s^n. For instance, if s = 0.99 and n = 20 days, the probability of surviving throughout the entire period is 81.8%, but only 9.9% if s is reduced by 10%. The parameter determining the shape of the functional response curve (β) is also very sensitive to small changes. Experimentally β is estimated to 1, which corresponds to a type II functional response. Increasing β by 10% (to 1.1) changes the functional response from a type II to a type III (a sigmoid curve) and the resulting *FlyDays* falls with 36% compared to the standard case. However, there is no experimental evidence pointing at β being significantly different from unity (Skovgård and Nachman 2015a).

8.5. Control methods of stable flies

We used the model to analyse three different control methods of stable flies: biological control by means of parasitoids, chemical control by means of a larvicide, and physical control by removal of manure. The three methods can be used either singly or in combinations. Skovgård (2004) estimated that biological control by means of *S. cameroni* will amount to US $1,944–$3,888 on a typical Danish farm, while the same farm should only invest US $984–$1968 if it bases fly control on the larvicide cyromazin. However, he suggested that the price of parasitoids can be more than halved if *S. cameroni* can be mass-reared on pupae of the black dump fly instead of house fly pupae. We have not attempted to estimate the costs (in terms of labour or money) associated with the various control strategies, so the different strategies cannot be compared with respect to cost efficiency. Furthermore, a cost-benefit analysis would require that the costs of control are weighted against the resulting economic benefits obtained by the control measures. Although stable flies undoubtedly cause some direct economic losses to the farmers in Denmark, the main reason for controlling them is that they are a nuisance to humans living on and around a farm. As we were unable to put an economic value on the advantage of avoiding stable flies, we limited our analysis to investigate the relationship between the control efforts on the one side (e.g. the number of treatments) and the outcome (i.e. the percent reduction in fly abundance) on the other side. The simulations therefore provide information about the marginal gains obtained by increasing the control efforts.

8.5.1. Control by removal of manure

The simulations demonstrate that removal of manure is a very efficient way of getting rid of flies. Actually, the simulations show that it is possible to eradicate stable flies by thorough and frequent removal of all manure, but only because we have regarded the stable as a closed system without access of flies from outside. However, it seems likely that adult stable flies both immigrate to and emigrate from the stable. Thus, Skovgård & Nachman (2012) estimated daily dilution and loss rates at the farm in Marke (Fig. 4.2), using a mark-recapture method, but the method does not permit estimation of the contribution of immigration and emigration to dilution and loss rate, respectively (Nachman & Skovgård 2012). Nevertheless, the rather high per capita rates of dilution (around 0.2 day^{-1}) means that we cannot rule out the possibility that there is some influx of adult flies from the surroundings, implying that stable flies are likely to be a recurrent problem after they have seemingly been eradicated.

Though it is quite evident that maintaining a high hygienic standard in livestock production could keep the flies at low densities, it is not always possible, neither economically or practically, to clean the livestock facilities as thorough and frequently as required, especially when cattle are housed in large free-stall compartments. It may therefore be necessary to supplement with chemical and/or biological control.

8.5.2. Control by means of parasitoids

In organic cattle farms, the use of pesticides is prohibited, so the only efficient supplement to sanitation is biological control. In Denmark, good results against house and stable flies have been achieved by means of inundative releases of *S. cameroni* on a number of organic farms (Skovgård 2004, Skovgård & Nachman 2004). Typically, 50-80 female parasitoids were released per m^2 every week from April to September-October (Skovgård & Nachman 2004). Abundance of flies was assessed by means of the visual index scale by Kristiansen & Skovmand (1985) and converted to the average number of flies per animal.

The Fly Simulator allows for simulating the release strategies used in the above-mentioned experiments. The experimental data are not directly comparable with the simulation output because fly densities in the former were expressed as adult flies per animal, while the simulation model predicts the size of the entire population of adult flies (which can be converted to flies per m^2 manure). As we do not know the proportion of time flies spend on animals, we cannot convert total number of flies to the average number of flies per animal and *vice versa*, but it seems reasonable to assume that the two numbers are proportionally related to each other so that temporal variations in flies per cattle can be used to express relative changes in abundances (see also Section 2.1.4).

When *The Fly Simulator* was applied to simulate pre-planned releases of parasitoids, we found that weekly releases of 9,259 parasitoids from mid-April to mid-October during two years (in total 500,000 individuals) was sufficient to suppress the stable fly population by 72% (Fig. 6.3). This corresponds to ca 93 parasitoids released per m^2 or, since 70.7% are females, to ca 66 females m^{-2}. This number agrees well with the release sizes applied by Skovgård & Nachman (2004).

An adaptive strategy based on releasing parasitoids every time the number of stable flies exceeds 10,000 adults yielded better results than a threshold of 20,000 individuals, but only when the time interval between releases is 1 week or longer (Fig. 7.7). However, when the time interval between releases is 2 weeks or more, it seems better to apply 20,000 stable flies as the threshold for intervention and then use 40,000 parasitoids per release. Thus, if the interval between two releases is at least 4 weeks, it is possible to obtain a *CI* of 62.2% by means of 12 releases of 40,000 parasitoids each, whereas *CI* based on 17 releases of 20,000 parasitoids is only 42.4%. In the former case, the total number of parasitoids is 480,000 and in the latter 340,000. In comparison, a preplanned release strategy based on a total release size of 480,000 parasitoids produced a *CI* of 62.3% if 34,286 parasitoids were released every 4 week from April 15 through October 15 in both years (14 releases).

Although this points at the adaptive strategy to be more cost-efficient than the preplanned strategy, this will probably not be the case if the cost of sampling and the risk of making wrong decisions based on uncertain information about population sizes are taken into account. Furthermore, the logistics associated with a pre-planned strategy with respect to deliveries and releases are simpler than an adaptive strategy, where the time and size of deliveries change from week to week. The main disadvantage of a pre-planned release strategy is that parasitoids are wasted if they are released at times where they may not be really needed. However, this can be regarded as a minor problem if the price of producing parasitoids can be reduced by applying large-scale mass production facilities. Thus, we expect that a pre-planned strategy based on releases of ca 400 parasitoids m^{-2} every four week during the fly season can be economically affordable and at the same time give a significant reduction in fly abundance (Fig. 7.4).

8.5.3. Control by means of insecticides

We analysed the efficiency of a specific product, the larvicide azadirachtin, because information on the dose-lethality relationship of this pesticide could be obtained from Miller and Chamberlain (1989). However, newer insecticides (both larvicides and adulticides) are now available at the market and can easily be incorporated into *The Fly Simulator* model as optional choices of pesticides, allowing the user to compare how different pesticides can be used optimally. We have refrained from making such an extension of the simple reason that we only wanted to demonstrate the contrast between controlling stable flies by means of parasitoids versus insecticides, and to be able to investigate how the two control methods supplement or counteract each other when used in combination as part of an IPM strategy.

In contrast to biological control based on natural enemies, pesticides can be easily stored and used immediately when needed. Furthermore, the extra mortality exerted by insecticides is likely to be density-independent (i.e. the same proportion of larvae are killed irrespective of larval density), whereas the parasitoids can only realize their attack potential when the density of fly pupae is high, and therefore give best results on farms where flies are abundant.

However, insecticides have some disadvantages, such as development of resistance, and risk of poisoning humans and non-target organisms, which means that the use of pesticides should be minimized. Our simulations, although based on a very premature data, indicate that it is possible to identify an application strategy that balances control of flies and the use of pesticides.

Unfortunately, we lack information about the speed at which azadirachtin is degraded in manure. The model assumes that the concentration of pesticide decreases with a constant rate which is independent of temperature and humidity. This is an obvious simplification as it is commonly known that degradation of pesticides in e.g. soil and water is temperature-dependent (see e.g. Wang et al. 2014). We assumed a degradation rate of 10% per day (corresponding to a 50% reduction of the initial concentration after less than 7 days), but a slower degradation rate implies that fewer applications will be needed.

The simulations demonstrate that stable flies can be significantly reduced by means of a larvacide and that both the dose and the interval between treatments affect its efficiency. We combined these two factors into a common measure called the cumulated pesticide dose (*CPD*), which expresses the total amount of pesticide used during a given period of time. Figure 7.14 shows that the reduction in fly abundance (expressed as *FlyDays*) increases with *CPD,* but levels out when *CPD* exceeds 30,000 ml, indicating that a further increase in the use of pesticides at best will be a waste of money but might even lead to poorer control, as indicated by Fig. 7.16. The likely explanation for this seemingly paradoxical result is that the larvicide reduces the larval density and thereby lessens the strong intraspecific competition among the larvae, which otherwise would cause high larval mortality (see also section 8.3). If such an antagonistic effect of a pesticide can also be evidenced experimentally, it is important to take it into consideration when stable flies, or maybe many other insect pests, are controlled by pesticides.

8.5.4. Integrated control

Although the costs and benefits associated with the different control strategies cannot be expressed in a common currency, the simulations indicate that the benefits obtained by using integrated control are not justified by the costs it imposes to the environment. The relatively small advantage in terms of *CI* score obtained by integrated control compared with biological control alone can be compensated by increasing the number of parasitoids used per release. For instance, pre-planned releases of 40,000 parasitoids every third week yield a *CI* of 81.1% while *CI* is 92.4% if 50,000 parasitoids are used.

Integrated pest management (IPM) of stable flies should also consider the advantages of maintaining a high sanitation standard in the production facilities. Thus, the simulations demonstrate that fly problems can be significantly reduced by removing manure at regular intervals. However, there will probably still be a few flies around, developing in small isolated pockets of manure. If biological control by means of natural enemies should work efficiently under these conditions, it would require that the enemy species has a high dispersal ability enabling it to quickly locate and exploit the immature flies occupying such pockets. The current version of *The Fly Simulator* is not able to cope with such shifting dynamics, also called "hide-and-seek" dynamics (see e.g. Nachman 2001), so we cannot use it to predict how efficient biological control by means of *S. cameroni* (or other enemies of flies) will be under these circumstances. If biological control against low density populations of flies does not work, insecticides will be the only option, because their efficiency does not depend on the density of flies, i.e. they kill a fixed proportion of the remaining flies. It should, however, be remembered that the marginal gains of using insecticides against low populations of pest are likely to be small compared with the costs because the dose needed to kill half of the population is the same irrespective of its density. Therefore, it is necessary to accept that stable flies cannot be eradicated so control should instead aim at keeping them at a tolerable level.

8.5.5. Strategic and tactical models and their applications in IPM

Computer-based simulation models are important tools in pest management, including flies (Lysyk & Axtell 1987, Wilhoit et al. 1991). Models can be used both as strategic and tactical tools in IPM (Conway 1984). This study has demonstrated how a simulation model can be used as a strategic tool for choosing between different control options. A strategic model can be used to investigate different scenarios and to answer questions like: What would have happened if the farmer had removed manure instead of releasing parasitoids, or is it better to remove manure instead of applying a larvicide? The

outcome of different scenarios can then be compared retrospectively with the actual outcome (in our case data from Marke without any control measures taken against stable flies). Unfortunately, we have only good data from a single farm, so the generality of the recommendations can of course be questioned. Nevertheless, the simulation model helps us to identify promising control strategies, which afterwards can be tested in practice, thereby avoiding a lot of trial-and-error experiments.

A strategic model provides some simple guidelines as how to manage a pest problem. In the case of stable flies the rules are for instance release *x* parasitoids every *y* week through a given period (a pre-planned strategy) or apply a larvicide when the density of stable flies exceeds *z* individuals per cow (an adaptive strategy). Such rules should be easy to implement by the farm operator and do not require any special skills.

A tactical model is more sophisticated and requires more knowledge to apply. It is based on combining current information about the densities of the pest and its natural enemies with reliable information about the expected weather conditions, so as to predict how the fly population will develop during the next one or two weeks. If the model predicts that the abundance of flies will exceed a given threshold, defined by the farm operator, some actions should be taken. The kind of action and its consequences can then be decided based on simulating different scenarios by means of the tactical model. Hence, a tactical approach to pest control requires that reliable information about current population sizes can be easily obtained and that success criteria for applying the different control options can be defined.

Counts of the number of flies per animal, using a simple visual index as suggested by Kristiansen & Skovmand (1985) and applied by e.g. Skovgård (2004), may be a good proxy for fly density in a stable (Section 2.1.4). Digital photos of selected parts of animals may serve the same purpose by making counting easier. Likewise, the percentage of parasitized house fly pupae placed in small sentinel mesh bags in selected areas of the animal facilities may provide information about the activity of parasitoids to be used as a proxy for their abundance (Skovgård & Nachman 2004, Skovgård 2006). Finally, a nuisance level of 13-15 house flies per animal has been used as a criterion for initiating treatment with insecticides (Skovgård 2004). However, it seems likely that the nuisance threshold, as well as the economic threshold, is lower when it comes to stable flies (see also Section 2.1.5).

9. Conclusions

Populations of stable flies have the potential to grow quickly and reach high densities in dairy cattle operations, provided the right conditions are available. In this study, we estimated the maximum per capita net growth rate (r_m) of *S. calcitrans* to be 0.090 per day which corresponds to a doubling time of 7.7 days. Although some authors have found even higher growth rates, our result agrees well with values found in field studies. We also found that populations of stable flies will be able to increase when the ambient temperature is in the range between 13 and 33°C with an optimum temperature around 26°C. Therefore, when stable temperatures start to rise in early spring we usually see severe outbreaks of stable flies in cattle operations with ample supply of well-conditioned manure. In conventional farms, such outbreaks can be prevented or mitigated by using insecticides as illustrated by means of model simulations, but on organic farms this will not be an option. Our simulations demonstrate that the best control strategy will be to deny the flies access to manure. However, if this is not sufficient, we have shown that biological control by means of *S. cameroni* can be an efficient way of controlling stable flies. On conventional farms, biological control can be combined with chemical control as part of an IPM strategy, although our simulations indicate that the advantage of using insecticides in combination with biological control is marginal.

Our data show that *S. cameroni* has a maximum per capita net growth rate (r_m) of 0.153 per day, which is achieved at 32.8°C. However, to realize such a high growth rate, the parasitoids need unlimited access to fly pupae, which will usually not be the case under field conditions. The functional response of *S. cameroni* was found to be a type II. The upper asymptote of the curve was found to be temperature dependent, so both the host density and the temperature affect the ability of parasitoids to attack hosts. Besides, mutual interference among the searching female parasitoids should be taken into consideration when the density of parasitoids is high. Maximum attack and oviposition rates per female were estimated to be 20.23 attacks d^{-1} and 18.20 ovipositions d^{-1}, respectively.

The model shows that *S. cameroni* requires temperatures above 22°C in order to maintain a persistent population. This means that the species cannot establish permanently in the stable system due to the low winter temperatures. We also found that the average temperature during summertime is too low to obtain satisfactory control of stable flies by means of a single inoculative release in spring or early summer. However, we found that good control can be achieved if *S. cameroni* is used in connection with inundative releases. For instance, if 400 adult parasitoids are released per m^2 every 4 week during the fly season (mid-April to mid-October), it is possible to obtain a significant reduction in stable flies. This result is supported by the field experiments conducted by Skovgård (2004) and Skovgård & Nachman (2004).

We expect that biological control of stable flies will become more common in the future, partly because the continued use of insecticides is problematic, and partly because neither the farmers nor their neighbours are willing to accept high densities of stable flies. Unfortunately, biological control is still too expensive compared with chemical control, but we expect that the former will become more competitive as more farmers switch from chemical to biological control.

10. Perspectives

In Denmark, stable flies are still considered as a minor problem in conventional dairy cattle operations because the flies can be adequately controlled by insecticides. However, there seems to be a growing demand both from the authorities and the consumers to avoid or reduce the use of insecticides in modern livestock production. It means that flies in the future are likely to become a much more serious nuisance, affecting not only the farmers and their families, but also their neighbours, unless the farmers take good care of cleaning the confinement facilities for manure. As this may not always be manageable, it is paramount to develop biological control as an effective alternative to insecticides. The simulation model and the data presented in this report have demonstrated that biological control by means of the parasitoid *S. cameroni* can be such an alternative, provided the parasitoids are released in large numbers and at several occasions so as to cope with the fast developing populations of flies during the fly season.

In this report, we have focused on the management of stable flies by means of *S. cameroni*, but we believe that the same principles applying to stable flies can be applied to other fly species, first of all the house fly (*M. domestica*). Although there are some differences between stable flies and house flies with respect to their biology and ecology, the two species share the same natural enemies, including *S. cameroni*, which makes it likely that house flies will be a smaller problem in stables where successful control of stable flies is achieved (Skovgård & Nachman 2004). We also suggest investigating whether better control of stable flies can be achieved if *S. cameroni* is supplemented by releases of other species of natural enemies as, e.g., *M. raptor* (Skovgård 2006). If natural enemies are produced commercially, it is necessary to ensure that the products satisfy a high quality standard with respect to species composition, number of individuals, sex ratio, viability etc. Otherwise, the end-users will lose confidence in biological control.

When we compared pre-planned and adaptive control strategies, they were regarded as two mutually exclusive strategies, but in practice they can be combined. Thus, a pre-planned release strategy based on, e.g., monthly releases of natural enemies can be seen as an insurance or protective shield against sudden outbreaks of flies. The method may work fine in most years, but in years with, e.g., unusual high summer temperatures, the pre-planned strategy can be supplemented with either releases of extra parasitoids or pesticide applications.

Seen in a wider perspective, the next step will be to develop *The Fly Simulator* from being a strategic research tool to become a tactical model to be used in a computer-aided support system available to farmers and advisors for on-farm management of fly problems (see Section 8.4.5).The most critical input to such a management system is information about the current population sizes of flies and their natural enemies. Reasonable precise estimates of total population size of adult flies can be obtained by a mark-recapture procedure, but as this is very time-consuming, there is a need of testing whether some simple fly indices (e.g. flies per animal) are acceptable proxies for the total population size. Research is also needed to investigate the relationship between sampling effort (e.g. sample size or time allocated for monitoring) and the precision of the estimated population size based on abundance indices. This will allow for incorporating sampling error (and probably also other sources of noise) into the simulations to predict best and worst case scenarios.

We hope that this report will stimulate interest among Danish famers to stop using insecticides against flies and instead switch to biological control, as this method seems to be the only long-term sustainable solution to the pest problem. However, in order for biological control to be successful it is necessary that the farmers become convinced that the method is as reliable as chemical control and, at the same time, is economically sound. In the transition period from chemical to biological control, it might be necessary that the farmers and/or the producers of natural enemies are offered economic support to keep the costs at the same level as chemical control. An alternative is to increase the taxes on insecticides and/or put some restrictions on their use. We also anticipate that the advisory service will become an important link between the researchers and the farmers to dissipate information about biological control. Finally, there might be a need for web-based training courses using photos and videos to convey information about the pest system and how to use natural enemies. Such information can be supplemented by computer “games” based on simulation programs like *The Fly Simulator*, where the player is supposed to be a farmer who has to take the right decisions when confronted with complex scenarios.

In the long term we expect that cost-efficient and environmentally sustainable pest management will require so much know-how that the individual farmers will see their advantage in handing over the responsibility for pest control to private companies specialized in producing, delivering and releasing natural enemies. This may not only give better fly control to the benefit of both animals and humans, but also allow the farmers to focus on how to produce high quality agricultural products.

References

Andress ER & Campbell JB 1994. Inundative releases of pteromalid parasitoids (Hymenoptera: Pteromalidae) for the control of stable flies, *Stomoxys calcitrans* (L.) (Diptera: Muscidae) at confined cattle installations in west central Nebraska. J. Econ. Entomol. 87: 714-722.

Axtell RC 1986. Fly control in confined livestock and poultry production. Technical Monograph, CIBA-GEIGY Corporation pp. 59.

Axtell RC & Rutz DA 1986. Miscellaneous publications of the Entomological Society of America. 61: 88-100.

Bailey NTJ 1952. Improvements in the interpretation of recapture data. J. Anim. Ecol. 21: 120-127.

Berkebile DR, Thomas GD & Campbell JB 1994. Overwintering of the stable fly (Diptera: Muscidae) in southeastern Nebraska. J. Econ. Entomol. 87: 1555-1563.

Berry IL, Kunz SE, & Foerster KW 1977. A dynamic model of the physiological development of immature stable flies. Ann. Entomol. Soc. Amer. 70:173-176.

Berry IL, Foerster KW, & Campbell JB 1978. Overwintering behaviour of stable flies in manure mounds. Environ. Entomol. 7: 67-72.

Birkemoe T, Soleng A, & Aak A 2009. Biological control of *Musca domestica* and *Stomoxys calcitrans* by mass releases of the parasitoid *Spalangia cameroni* on two Norwegian pig farms. BioControl 54: 425-436.

Birkemoe T Soleng A & Skovgård H 2012. Life history parameters of two geographically separated populations of *Spalangia cameroni*, a microhymenopteran pupal parasitoid of muscoid flies. BioControl 57: 375-385.

Bouček Z 1963. A taxonomic study in *Spalangia* Latr. (Hymenoptera, Chalcidoidae). Acta Ent. Musei National Prague, 35: 430-512.

Briggs CJ, Murdoch WW & Nisbet RM 1999. Recent developments in theory for biological control of insect pests by parasitoids. *In* B.A. Hawkins and H.V. Cornell (eds). Theoretical Approaches to Biological Control. Cambridge University Press, Cambridge.

Broce AB, Hogsette J, & Pailey S 2005. Winter feeding sites of hay in round bales as major developmental sites of *Stomoxys calcitrans* (Diptera: Muscidae) in pastures in spring and summer. J. Econ. Entomol. 98: 2307-2312.

Bruce WN, & Decker GC 1958. The relationship of stable fly abundance to milk production in dairy cattle. J. Econ. Entomol. 51: 269-274.

Böckmann EA Tormos J Beita F Fischer K 2012. Offspring production and self-superparasitism in the solitary ectoparasitoid *Spalangia cameroni* (Hymenoptara: Pteromalidae) in relation to host abundance. Bull. Ent. Res. 102: 131-137.

Campbell JB, White RG, Wright JE, Crookshand R, & Clanton DC 1977. Effects of stable flies (Diptera: Muscidae) on weight gains and feed efficiency of calves on growing or finishing rations. J. Econ. Entomol. 70: 592-594.

Campbell JB, Skoda SR, Berkebile DR, Boxler DJ, Thomas GD, Adams DC, & Davis R. Effects of stable flies (Diptera: Muscidae) on weight gain of grazing yearling cattle. J. Econ. Entomol. 94: 780–783.

Catangui MA, Campbell JB, Thomas GD, & Boxler DJ 1997. Calculating economic injury levels for stable flies (Diptera: Muscidae) on feeder heifers. J. Econ. Entomol. 90: 6-10.

Cilek JE, Greene GL 1994. Stable flies (Diptera, Muscidae) insecticide resistance in Kansas cattle feedlots. J. Econ. Entomol. 87: 275–279.

Clemson B, Tang Y, Pyne J & Unal R 1995. Efficient methods for sensitivity analysis. Systems Dynamics Rev. 11: 31-49.

Cranshaw W, Sclar DC & Cooper D 1995. A review of 1994 pricing and marketing by suppliers of organisms for biological control of arthropods in the United States. Biological Control 6: 291-296.

Conway GR 1984. Pest and Pathogen Control. Strategic, Tactical, and Policy Models. John Wiley & Sons. Chichester NY.

Dansk Landbrug i tal. 2009. Udgivet af Landbrug & fødevarer, Axelborg, København.

Dansk Kvæg 2004. Kvægbruget i tal. www.netbutikken.landcentret.dk

DeBach P 1964. Biological control of insect pests and weeds. New York: Reinhold Publishing Corp.

DeBach P, & Rosen D 1991. Biological control by natural enemies. Cambridge University Press.

Floate KD, & Fox AS 1999. Indirect effects of ivermectin residues across trophic levels: *Musca domestica* (Diptera: Muscidae) and *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae). Bull. Ent. Res. 89: 225-229.

Floate KD, & Skovgård H 2004. Winter survival of nuisance fly parasitoids (Hymenoptera: Pteromalidae) in Canada and Denmark. Bull. Entomol. Res. 94:331-340.

Floate KD, P. Coghlin, & GAP Gibson 2000. Dispersal of the filth fly parasitoid *Muscidifurax raptorellus* (Hymenoptera: Pteromalidae) following mass releases in cattle confinements. Biological Control 18: 172-178.

Freeborn SB, Regan WM, & Folger AH 1928. The relation of flies and fly spray to milk production. J. Econ. Entomol. 18: 779-790.

Friesen KM, & Johnson GD 2012. Reproductive Potential of Stable Flies (Diptera: Muscidae) Fed Cattle, Chicken, or Horse Blood. J. Med. Entomol. 49: 461-466.

Förster M, Sievert K, Messler S, Klimpel S, and Pfeffer K 2009. Comprehensive study on the occurrence & distribution of pathogenic microorganisms carried by synanthropic flies caught at different rural locations in Germany. J. Med. Entomol. 46: 1164-1166.

Gerling D and Legner EF 1968. Development history & reproduction of *Spalangia cameroni*, parasite of synanthropic flies. Ann. Ent. Soc. Am. 61: 1436-1443.

Geden CJ 1996. Modeling host attacks & progeny production of *Spalangia gemina*, *Spalangia cameroni*, and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) at constant & variable temperatures. Biological Control 7: 172-178.

Geden CJ 1997. Development models for the filth fly parasitoids *Spalangia gemina*, *S. cameroni*, and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) under constant and variable temperatures. Biological Control 9: 185-192.

Geden CJ 2002. Effect of habitat depth on host location by five species of parasitoids (Hymenoptera: Pteromalidae, Chalcidiae) of house flies (Diptera: Muscidae) in three types of substrates. Environ. Entomol. 31: 411-417.

Geden CJ & Axtell RC 1988. Predation by *Carcinops pumilio* (Coleoptera: Histeridae) and *Macrocheles muscaedomesticae* (Acarina: Macrochelidae) on the house fly (Diptera: Muscidae): Functional response, effects of temperature, and availability of alternative prey. Environ. Entomol. 17: 739-744.

Gilbert N & Gutierrez AP 1973. A plant-aphid-parasitoid relationship. J. Anim. Ecol. 42: 323-340.

Gilles J, David J-F & Duvallet G 2005a. Temperature effects on development and survival of two stable flies, *Stomoxys calcitrans* and *Stomoxys niger niger* (Diptera: Muscidae), in La Réunion Island. J. Med. Entomol. 42: 260-265.

Gilles J, David J-F & Duvallet G 2005b. Effects of temperature effects on the rate of increase of *Stomoxys calcitrans* and *Stomoxys niger niger* (Diptera: Muscidae) from La Réunion Island. J. Med. Entomol. 42: 959-965.

Greene GI 1989. Seasonal population trends of adult stable flies, pp. 12-17. *In*. JJ Petersen & GL Greene (*eds*), Proceedings, Symposium: Current status of stable fly (Diptera: Muscidae) research, National conference of the entomological society of America, December 1986, Reno, Nevada. Miscellaneous publications 74, Entomological Society of America.

Gregor F, Rozkošný R, Barták M, andVaňhara J 2002. The Muscidae (Diptera) of Central Europe. Masaryk University, Brno, Czech Republic pp. 280.

Grimm V & Railsback SF (2005). Individual-based Modeling and Ecology. Princeton University Press, Princeton, NJ.

Hald B, Skovgård H, Pedersen K, & Bunkenborg H. 2008. Influxed insects as Vectors for *Campylobacter jejuni* and *Campylobacter coli* in Danish Broiler Houses. *Poultry Science* 87: 1428-1434.

Harmsen K 2000. A modified Mitscherlich equation for rainfed crop production in semi-arid areas: 1. Theory. Netherlands Journal of Agricultural Science 48: 237-250.

Hassell MP 1978. The dynamics of arthrop predator-prey systems. Monographs in Population Biology 13.Princeton University Press, Princeton, NJ.

Hoddle M, van Driesche R & Sanderson J 1997. Biological control of *Bemesia argentifolii* (Homoptera: Aleyrodidae) on *Poinsetta* with inundative releases of *Encarsia formosa* (Hymenoptera: Aphelinidae): Are higher release rates necessarily better? Biol. Contr. 10: 166-197.

Hogsette JA, Ruff, JP, and Jones CJ 1989. Dispersal behavior of stable flies (Diptera: Muscidae), pp. 23-32. *In*. JJ Petersen & GL Greene (*eds*), Proceedings, Symposium: Current status of stable fly (Diptera: Muscidae) research, National conference of the entomological society of America, December 1986, Reno, Nevada. Miscellaneous publications 74, Entomological Society of America.

Holling CS 1959. Some characteristics of simple types of predation and parasitism. Can. Entomol. 91: 385-398.

Ivlev VS 1961. Experimental ecology of the feeding of fishes. Yale University Press, New Haven, Connecticut, USA.

Jacquiet P, Rouet D, Bouhsira E, Salem A, Lienard E, & Franc M 2014. Population dynamics of *Stomoxys calcitrans* (L.) (Diptera: Muscidae) in southwestern France. Revue de Medecine Veterinaire 165: 267-271.

Johns GG & Vimpany JA 1999. Interaction of pH amendment and potassium fertilizer on soil chemistry and banana plant growth. Australian Journal of Agricultural Research, 50: 199-210.

Kaufman PE, Rutz DA, & Frisch S 2005. Large sticky traps for capturing house flies and stable flies in dairy calf greenhouse facilities. J. Dairy Sci. 88: 176-181.

Kaufman PE Strong C Waldron JK & Rutz DA 2012. Individual and combined releases of *Muscidifurax raptor* and *M. raptorellus* (Hymenoptera: Pteromalidae) as a biological control tactic targeting house flies in dairy calf facilities J. Medical Entomol. 49: 1059-1066.

Keiding J 1999. Review of the global status and recent development of insecticide resistance in field populations of the house fly, *Musca domestica* (Diptera: Muscidae). Bull. Entomol. Res. 89: s7-s67.

King BH 1997. Effects of age and burial of house fly (Diptera: Muscidae) pupae on parasitism by *Spalangia cameroni* and *Muscidifurax raptor* (Hymenoptera: Pteromalidae) Environ. Entomol. 26: 410-415.

Kristiansen K & Skovmand O 1985. A method for the study of population size and survival rate of house flies. Entomol. Exp. Appl. 38: 145-150.

Kunz SE, Berry IL, & Foerster KW 1977. The development of the immature forms of *Stomoxys calcitrans*. Ann. Entomol. Soc. Amer. 70: 169-172.

Larsen EB & Thomsen M 1941. The influence of temperature on the development of some species of Diptera. Vidensk. Medd. fra Dansk naturh. Foren. 104: 1-75.

Larsen D 2006. A stage structured simulation model based on the interaction between the parasitoid *Spalangia cameroni* and its host *Stomoxys calcitrans,* a nuisance pest in animal stables. Unpublish M.Sc. thesis at University of Copenhagen.

Legner EF 1967. Behavior changes the reproduction of *Spalangia cameroni*, *S. endius*, *Muscidifurax raptor*, and *Nasonia vitripennis* (Hymenoptera: Pteromalidae) at increasing fly host densities. Ann. Entomol. Soc. Amer. 60: 819-826.

Legner EF 1978. Muscidae, pp. 346-355. *In*. Clausen CP (ed), Introduced parasites and predators of arthropod pests and weeds: a world review. United States Department of Agriculture, Agriculture Handbook No. 480, Washington. Pp. 545.

Legner EF, EC Bay, & White EB 1967. Activity of parasites from Diptera: *Musca domestica*, *Stomoxys calcitrans*, *Fannia canicularis*, and *F. femuralis*, at sites in the western hemisphere. Ann. Entomol. Soc. Amer. 60: 462-468.

Lysyk TJ 1993. Seasonal abundance of stable flies and house flies (Diptera: Muscidae) in dairies in Alberta, Canada. J. Med. Entomol. 30: 888-895.

Lysyk TJ 1998. Relationships between temperature and life-history parameters of *Stomoxys calcitrans* (Diptera: Muscidae). J. Med. Entomol. 35: 107-119.

Lysyk TJ, & Axtell RC 1987. A simulation model of house fly (Diptera: Muscidae) development in poultry manure. Can. Ent. 119: 427-437.

Lysyk TJ & Schaalje GB 1992. Binomial sampling for pest management of stable flies (Diptera: Muscidae) that attack dairy cattle. J. Econ. Entomol. 85: 136-?

Mann JA, Axtell RC, & Stinner RE 1990. Temperature-dependent development and parasitism rates of four species of Pteromalidae (Hymenoptera) parasitoids of house fly (*Musca domestica*) pupae. Med. Vet. Entomol. 4: 245-253.

May RM 1974. Model Ecosystems. Monographs in population biology 6. Princeton University Press, Princeton, New Jersey.

Mckay T, & Galloway TD 1999. Survey and release of parasitoids (Hymenoptera) attacking house and stable flies (Diptera: Muscidae) in dairy operations. Canadian Entomol. 131: 743-756.

Meyer JA, & Petersen JJ 1983. Characterization and seasonal distribution of breeding sites of stable flies and house flies (Diptera: Muscidae) on eastern Nebraska feedlots and dairies. J. Econ. Entomol. 76: 103-108.

Miljøstyrelsen 2011. Miljøstyrelsens Bekæmpelsesmiddel statistik.

Miller JA & Chamberlain WF 1989. Azadirachtin as a larvicide against the horn fly, stable fly, and house fly (Diptera: Muscidae). J. Econ. Entomol. 82: 1375-1378.

Mironidis GK & Savopoulou-Soultani M 2008. Development, survivorship, and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae) under constant and alternating temperatures. Physiol. Ecol.37: 16-28.

Moon RD, Berry IL & Petersen JJ 1982. Reproduction of *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae) on stable fly (Diptera: Muscidae) in the laboratory. J. Kansas Entomol. Soc. 55: 77-85.

Mourier H, & ben Hannine S 1971. Survey of the importance of natural enemies of houseflies in Denmark. Danish Pest Infestation Laboratory Annual Report, 1970, pp. 51-52.

Morgan PB 1981. The potential use of of parasites to control *Musca domestica* L. and other filth breeding flies at agricultural installations in the southern Unites States, pp. 11-25. *In*: Status of biological control of filth flies. U.S. Dept. Agric. Sci. Educ. Adm. Publ. A106.2:F64.

Morgan PB, Weidhaas DE & Patterson RS 1981. Host-parasite relationship: augmentative releases of *Spalangia endius* Walker used in conjunction with population modelling to suppress field populations of *Musca domestica* L. (Hymenoptera: Pteromalidae and Diptera: Muscidae). J. Kansas Ent. Soc. 54: 496-504.

Mueller LD & Joshi A 2000. Stability in model populations. Monographs in population biology 31. Princeton University Press, Princeton, New Jersey.

Mullens BA, Meyer JA & Mandeville JD 1986. Seasonal and diel activity of filth fly parasites (Hymenoptera: Pteromalidae) in caged-layer poultry manure in southern California. Environ. Entomol. 15: 56-60.

Mullens BA, Hinkle NC, & Szijj CE 1996. Impact of alternating manure removal schedules on pest flies (Diptera: Muscidae) and associated predators Coleoptera: Histeridae, Staphylinidae; Acarina: Machrochelidae) in caged-layer poultry manure in southern California. J. Econ. Entomol. 89: 1406-1417.

Mullens BA, & Peterson NG 2005. Relationship between rainfall and stable fly (Diptera: Muscidae) abundance on California dairies. J. Med. Entomol. 42: 705-708.

Mullens BA, Lii KS, Mao Y, Meyer JA, Petersen NG, & Szijj CE 2006. Behavioural responses of dairy cattle to the stable fly, *Stomoxys calcitrans* in an open field environment. Med. Vet. Entomol. 20: 122-137.

Nachman G 2001. Predator-prey interactions in a nonequilibrium context: the metapopulation approach to modelling "hide-and-seek" dynamics in a spatially explicit tri-trophic system. Oikos 94: 72-88.

Nachman G & Skovgård H 2012. A simulation study of how simple mark-recapture methods can be combined with destructive subsampling to facilitate surveys of flying insects. Env. Entomol. 41: 141-151.

Nachman G & Gotoh T 2015. Modeling the effects of constant and variable temperatures on the vital rate of an age-, stage, and sex-structured population by means of the SANDY approach. Env. Entomol. 44: 821-834.

Nicholson AJ 1954a. Compensatory reactions of populations to stresses, and their evolutionary significance. Austral. J. Zool. 2: 1-8.

Nijland GO, Schouls J & Goudriaan J 2008. Integration the production functions of Liebig, Michaelis-Menten, Mitscherlich andLiebscher into one system dynamics model. Netherlands Journal of Agricultural Science, 55: 200-224.

Nisbet RM & Gurney WSC 1982. Modelling fluctuating populations. John Wiley & Sons.

Nolan MP, & Kissam JB 1985. *Ophyra aenescens*: A potential bio-control alternative for house fly control in poultry houses. Agric Entomol. 2: 192-195.

Petersen JJ Catangui MA & Watson DW 1991. Parasitoid-induced mortality of house fly pupae by pteromalid wasps in the laboratory. Biological Control 1: 275-280.

Pitzer JB, Kaufman PE, & TenBroeck SH 2010. Assessing permethrin resistance in the stable fly (Diptera: Muscidae) in Florida using laboratory selections and field evaluations. J. Econ. Entomol. 103: 2258-2263.

Pitzer JB, PE Kaufman, CJ Geden, & Hogsette JA 2011. The ability of selected pupal parasitoids (Hymenoptera: Pteromalidae) to locate stable fly hosts in a soiled equine bedding substrate. Environ. Entomol. 40: 88-93.

Quicke, DLJ 1997. *Parasitic Wasps*. Chapman & Hall, London.

Rasmussen RL, & Campbell JB 1981. Investigation of environmental factors and their relationship to populations of the stable fly, *Stomoxys calcitrans* (L.). Environ. Entomol. 10: 798-800.

Régnière J & Logan, JA 2003. Animal life cycle models. In: *Phenology: An Integrative Environmental Science* (ed. M.D. Schwartz). Klüwer Academic Publishers, Dordrecht. pp. 237-254.

Régnière J, Powell J, Bentz B & Nealis V 2012. Effects of temperature on development, survival and reproduction of insects: Experimental design, data analysis and modeling. J. Insect Phys. 58: 634-647.

Rueda LM & Axtell RC 1985. Guide to common species of pupal parasites (Hymenoptera: Pteromalidae) of the house fly and other muscoid flies associated with poultry and livestock manure. North Carolina Agricultural Research Service Technical Bulletin 278, 88 pp.

Rueda LM & Axtell RC 1985. Effect of depth of house fly pupae in poultry manure on parasitism by six species of pteromalidae (Hymenoptera). J. Entomol. Sci. 20: 444-449.

Rutz DA, & Geden CJ 2000. Pest management recommendations for dairy cattle. A Cornell and Pennsylvania State University extension publication.

Salem A, Franc M, Jacquiet P, Bouhsira E & Liénard E 2012. Feeding and breeding aspects of *Stomoxys calcitrans* (Diptera: Muscidae) under laboratory conditions. Parasite 19: 309-317.

Schaalje GB & van der Vaart HR 1989. Relationships among recent models for insect population dynamics with variable rates of development. J. Math. Biol. 27: 399-428.

Schofield S & Torr SJ 2002. A comparison of the feeding behavior of tsetse and stable flies. Med. Vet. Entomol. 16: 177-185.

Scholl PJ 1986. Field population studies of *Stomoxys calcitrans* (L.) in eastern Nebraska. Southwest. Entomol. 11: 155-160.

Scholl PJ, Petersen JJ, Stage DA, & Meyer JA 1981. Open silage as an overwintering site for immature stable flies in eastern Nebraska. Southwest. Entomol 6: 253-258.

Seber GAF 1973. The estimation of animal abundance and related parameters. Griffin, London, United Kingdom.

Skovgård H & Jespersen JB 1999. Activity and relative abundance of hymenopterous parasitoids that attack puparia of *Musca domestica* and *Stomoxys calcitrans* (Diptera: Muscidae) on confined pig and cattle farms in Denmark. Bull. Ent. Res. 89: 263-269.

Skovgård H & Jespersen JB 2000. Seasonal and spatial activity of hymenopterous pupal parasitoids (Pteromalidae and Ichneumonidae) of the house fly (Diptera: Muscidae) on Danish pig and cattle farms. Environ. Entomol. 29: 630-637.

Skovgård H & Steenberg T 2001. Activity of pupal parasitoids of the stable fly *Stomoxys calcitrans* and prevalence of entomopathogenic fungi in the stable fly and the house fly *Musca domestica* in Denmark. BioControl 47: 45-60.

Skovgård H 2004. Sustained releases of the pupal parasitoid Spalangia cameroni (Hymenoptera: Pteromalidae) for control of house flies, Musca domestica and stable flies Stomoxys calcitrans (Diptera: Muscidae) on dairy farms in Denmark. Biol. Contr. 30: 288-297.

Skovgård H & Nachman G 2004. Biological control of house flies *Musca domestica* and stable flies *Stomoxys calcitrans* (Diptera: Muscidae) by means of inundative releases of *Spalangia cameroni* (Hymenoptera: Pteromalidae). Bull. Entomol. Res. 94: 555-567.

Skovgård H. 2006. Search efficiency of *Spalangia cameroni* and *Muscidifurax raptor* on *Musca domestica* pupae in dairy farms in Denmark. BioControl 51: 49-64.

Skovgård H & Nachman G 2012. Population dynamics of stable flies *Stomoxys calcitrans* (Diptera: Muscidae) on an organic dairy farm in Denmark based on mark-recapture with destructive sub-sampling. Env. Entomol. 41: 20-29.

Skovgård H & Nachman G 2015a. Temperature-dependent functional response of *Spalangia cameroni* (Perkins) (Hymenoptera: Pteromalidae), a parasitoid of *Stomoxys calcitrans* (L.) (Diptera: Muscidae). Environ. Entomol. 44: 90-99.

Skovgård H & Nachman G 2015b. Effect of Mutual Interference on the Ability of *Spalangia cameroni* (Hymenoptera: Pteromalidae) to Attack and Parasitize Pupae of *Stomoxys calcitrans* (Diptera: Muscidae). Environ. Entomol. 44: 1076-1084.

Skovgård H & Nachman G (submitted). Temperature- and Age-dependent Survival, Developmental and Oviposition Rates of the Pupal Parasitoid *Spalangia cameroni*.

Smith L & Rutz DA 1991. Microhabitat associations of hymenopterous parasitoids that attack house fly pupae at dairy farms in central New York. Environ. Entomol. 20: 675-684.

Smith TM & Smith RL 2009. Elements of Ecology. Pearson Education, San Francisco.

Somme L 1959. On the number of stable flies and house flies on Norwegian farms. Nor. Entomol. Tidskr. 11: 7-15.

Son Y & Lewis EE 2005. Modelling temperature-dependent development and survival of *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). Agric. Forest Entomol. 7: 201-209.

Sutherland B 1979. Some effects of temperature on the adults, eggs and pupae of *Stomoxys calcitrans* Linnaeus (Diptera: Muscidae). Onderstepoort J. vet. Res. 46: 223-227.

Taylor DB, Berkebile DR, & Scholl PJ 2007. Stable flies population dynamics in eastern Nebraska in relation to climatic variables. J. Med. Entomol. 44: 765-771.

Taylor DB, Moon RD, & Mark DR 2012. Economic impact of stable flies (Diptera: Muscidae) on dairy and beef cattle production. J. Med. Entomol. 49: 198-209.

Thomsen T 1938. Stuefluen (*Musca domestica*) og stikfluen (*Stomoxys calcitrans*). Undersøgelser over biologi og bekæmpelse samt en oversigt over andre til husdyr eller boliger knyttede fluearter. Copenhagen, (Denmark). Den Kongelige Veterinære Landbohøjskole, Landsøkonomiske Forsøgslaboratorium, København, AG Bangs forlag.

Titchener RN, Newbold JW, & Wright CL 1981. Flies associated with cattle in south west Scotland during the summer months. Res. Vet. Sci. 30: 109-113.

Van Straalen NM 1983. Physiological time and time-invariance. Journal of Theoretical Biology, 104: 349-357.

Zumpt F 1973. The Stomoxine biting flies of the world, Diptera: Muscidae. *Taxonomy, biology, economic importance and control measures*. Gustav Fischer Verlag, Jena pp. 175.

Wagner TL, Wu H-I., Sharpe PJH & Coulson RN 1984. Modeling distributions of insect development time: A literature review and application of the Weibull function. Annals of the Entomological Society of America, 77: 475-487.

Wang HZ, Zuo HG, Ding YJ, Miao SS, Jiang C & Yang H 2014. Biotic and abiotic degradation of pesticide Dufulin in Soilds. Environ. Sci. Pollut. Res. 21: 4331-4342.

Weibull, W. (1951). A statistical distribution of wide applicability. Journal of Appled Mechanics: 293-297.

Wieman GA, Campbel JB, Deshazer JA, & Berry IL 1992. Effects of stable flies (Diptera: Muscidae) and heat stress on weight gain and feed efficiency of feeder cattle. J. Econ. Entomol. 85: 1835-1842.

Wilhoit LR, Stinner RE & Axtell RC 1991. Computer simulation model of house fly management in confined-animal production systems. North Carolina Agricultural Research Service. Technical bulletin 296. Raleigh NC.

Williams DF 1973. Sticky traps for sampling populations of *Stomoxys calcitrans*. J. Econ. Entomol. 66: 1279-1280.

Appendix 1: Mathematical description of the model

A1.1. Overall model structure

The host and parasitoid populations are modeled as sex-, stage- and age-structured. As a starting point and to speed up the simulations, survival, fecundity, sex distribution and transition from one stage to the next are modeled as deterministic processes.

The host population (*Stomoxys calcitrans*) is divided into four stages: eggs, larvae, pupae and adults. At a given instant of time t, the number of hosts in these four stages are denoted H_1, H_2, H_3 and H_4, respectively, or generally as H_s, where s is the stage number (i.e. s =1, 2, 3, 4). The parasitoid population (*Spalangia cameroni*) consists of two stages: immatures and adults. Immature parasitoids comprise the three stages developing inside parasitized host pupae (i.e. eggs, larvae and pupae). The numbers of immature and adult parasitoids are denoted P_1 and P_2, respectively.

In the following, H and P are used as symbols for number of host and parasitoid individuals when it is important to distinguish between the two populations; otherwise N is used as a common symbol for number of individuals.

Each stage is divided into a number of age classes. An individual has age 0 when it enters a new stage and for each time step (Δt = 1 day) it spends in the stage, its age is updated with one day until the individual either moves to another stage or dies. Thus, for each time step an individual may either survive (with probability p_s) or die (with probability 1-p_s). Provided it survives, it may either hatch to the next state (with probability p_h) or remain in the same stage (with probability 1-p_h).

Mathematically, the dynamics of a population at time t can be written as

$$N(g,s,x) = p_s(g,s,x-1)\big(1-p_h(g,s,x-1)\big)N(g,s,x-1) \tag{A1.1}$$

where $N(g,s,x)$ denotes the expected number of individuals of gender g belonging to stage s and of age x ($x \geq 1$).

$p_s(g,s,x\text{-}1)(1\text{-}p_h(g,s,x\text{-}1))$ is the proportion of individuals in the previous age class that neither dies nor hatches during the time step, while $p_s(g,s,x\text{-}1)p_h(g,s,x\text{-}1)$ is the proportion that survives and hatches to the next stage.

The expected number of individuals entering stage s ($s > 1$) at time t is found as

$$N(g,s,0) = \sum_{x=1}^{\infty} p_s(g,s-1,x-1)\,p_h(g,s-1,x-1)N(g,s-1,x-1) \tag{A1.2}$$

The expected number of males (g = 1) and females (g = 2) entering the first stage (i.e. s = 1) are obtained as

$$N(1,1,0) = \sum_{x=1}^{\infty}\big(1-p_f(x-1)\big)F(x-1)N(2,S,x-1) \tag{A1.3a}$$

$$N(2,1,0) = \sum_{x=1}^{\infty} p_f(x-1)F(x-1)N(2,S,x-1) \tag{A1.3b}$$

where S denotes the last stage (adults), $F(x\text{-}1)$ is the mean fecundity of females of age x-1 and $p_f(x\text{-}1)$ is the proportion of female offspring produced by females in the age class.

The model consists of five sub-models: (1) The fly model; (2) The parasitoid model; (3) The interaction model, (4) The manure model; and (5) The pesticide model.

A1.2. The limiting factors concept

p_s, p_h, p_f and F are specific for a given gender, stage and age-class of the species in focus and may depend on the ambient environment as well as on the conditions to which individuals have been exposed earlier in their life. We assume that each of these variables has a maximum for an optimum combination of the factors that simultaneously affect the respective response variable. To illustrate this, let y denote the value of a given response function and let y_{max} be the maximum value

y takes if all n factors affecting y were optimal. In accordance with the multiplicative Mitscherlich model (Johns and Vimpany 1999, Harmsen 2000, Nijland et al. 2008) we write y as a product of limiting factors

$$y = y_{\max} f_1(\cdot) f_2(\cdot) \cdots f_n(\cdot) = y_{\max} \prod_{j=1}^{n} f_j(\cdot) \tag{A1.4}$$

where $f_j(\cdot)$ is the effect of the jth factor on y. $f_j(\cdot)$ can take values between 1 and 0. Thus, if $f_j(\cdot) = 1$ for all n factors, the maximum value of y is attained, whereas if one or more values of $f_j(\cdot)$ are < 1, y will be smaller than $y_{\max}$. Finally, y will be 0 if just one of the functions is 0.

The factors affecting y could be, e.g., the ambient temperature, humidity, amount and quality of food, the influence of conspecifics, competitors or natural enemies. $f(\cdot)$ can also be used to represent the effect of an individuals's age on y. In arthropods, aging is typically associated with ambient temperature, so instead of using the *chronological age* (x) of individuals, we may apply their *biological age*. The biological age of an individual with chronological age x is denoted $Q(x)$. It represents the cumulated effect of temperature experienced by an individual until age x (Régnière and Logan 2003) and is calculated as

$$Q(x) = \sum_{i=0}^{x-1} \Delta Q_i \tag{A1.5}$$

where ΔQ_i denotes the increase in biological age while the individual belonged to age class i (i = 0, 1, 2,.., x-1). If $Q_{\max}$ denotes the maximum biological age an individual can attain before it either completes the life stage or dies, $Q(x)/Q_{\max}$ is a value between 0 and 1, which corresponds to the *physiological age* (van Straalen 1983, Régnière et al. 2012).

Due to variation among individuals with respect to developmental rate, not all individuals entering a given stage will leave the stage again at the same time (Wagner et al. 1984). The distribution of developmental times is model by assuming that the proportion of individuals still in the stage which complete development during Δt and enter the next stage will increase as Q approaches $Q_{\max}$, implying that the mean developmental time will be shorter than the maximum (see e.g. Wagner 1984, Son & Lewis 2005).

We apply the SANDY model (Nachman & Gotoh 2015) as a general model for $f(\cdot)$. It is given as

$$f(V) = C\left(\frac{V - V_{\min}}{V_{\max} - V_{\min}}\right)^a \left(\frac{V_{\max} - V}{V_{\max} - V_{\min}}\right)^b \tag{A1.6}$$

where V denotes the actual value of a factor affecting f. $f(V)$ takes values be between 0 and 1 when $V_{\min} < V < V_{\max}$ and is 0 if $V \le V_{\min}$ or $V \ge V_{\max}$. a and b are two positive parameters that determine the shape of the function, and C is a constant that scales $f(V)$ to ensure that $0 \le f(V) \le 1$. C is computed as

$$C = \left(\frac{a}{a+b}\right)^{-a} \left(\frac{b}{a+b}\right)^{-b} \tag{A1.7}$$

if both a and $b > 0$, otherwise $C = 1$. If both a and $b > 0$, the function has a maximum (equal to 1) when $V_{opt} = \frac{aV_{\max} + bV_{\min}}{a+b}$. Otherwise, the maximum is located at $V_{\text{opt}} = V_{\min}$ when $a = 0$ and $b > 0$, and at $V_{\text{opt}} = V_{\max}$ when $a > 0$ and $b = 0$. If both a and b are 0, $f(V)$ will be equal to 1 for all $V_{\min} < V < V_{\max}$, and either 0 or 1 outside this range, depending on what process $f(V)$ represents.

In the following we will use the notation $\mathbf{S}(V)$ when $f(\cdot)$ is modelled by the SANDY model, i.e.

$$\mathbf{S}(V) = C\left(\frac{V - V_{\min}}{V_{\max} - V_{\min}}\right)^a \left(\frac{V_{\max} - V}{V_{\max} - V_{\min}}\right)^b \tag{A1.8a}$$

and the notation $\mathbf{S}(y,V)$ as a short-cut for

$$\mathbf{S}(y,V) = y_{\max}\mathbf{S}(V) = y_{\max} C\left(\frac{V - V_{\min}}{V_{\max} - V_{\min}}\right)^a \left(\frac{V_{\max} - V}{V_{\max} - V_{\min}}\right)^b \tag{A1.8b}$$

where y is the modelled variable and V the value of the factor affecting y. y_{max}, V_{min}, V_{max}, a and b are the specific parameters associated with the relationship. We call $\mathbf{S}(V)$ the Sandy transformation of V.

A1.3. Modeling survival and development of immature stages

Q at age x is found by means of Eqn A1.5, where the increase in biological age during the ith age interval when the ambient temperature is T is calculated as

$$\Delta Q_i(T) = \mathbf{S}(q,T)\Delta t = q_{max}\mathbf{S}(T) \tag{A1.9}$$

where q is the increase rate in Q at temperature T, i. e., $q = \mathbf{S}(T) = \left(\frac{T - T_{min}}{T_{max} - T_{min}}\right)^a \left(\frac{T_{max} - T}{T_{max} - T_{min}}\right)^b$. We set q_{max} to unity at the optimum temperature, i.e. when $T = T_{opt} = \frac{aT_{max} + bT_{min}}{a+b}$, which means that $\Delta Q_i = \Delta t$ at $T = T_{opt}$. $Q(x)$ will therefore be equal to the chronological age (x) when the ambient temperature (T) is equal to T_{opt} or otherwise $Q(x)$ will be $< x$. ΔQ_i will be 0 if $T \le T_{min}$ or $T \ge T_{max}$.

Survival rates of eggs, larvae and pupae are assumed to be independent of their age but to depend on the ambient temperature (T). Thus, the likelihood that an individual survives from day t to day $t+\Delta t$ is modeled as

$$s(T) = \mathbf{S}(s,T) = s_{max}\mathbf{S}(T) \tag{A1.10}$$

where the parameters of the SANDY transformation depend on species, gender and stage.

The likelihood that an individual hatches from one stage to the next during Δt depends on Q. As hatching is 0 when $Q/Q_{max} = 0$, we may use Eqn A1.6 to model hatch probability (h) by replacing V with Q, V_{min} with 0 and V_{max} with Q_{max}, yielding

$$h(Q) = \mathbf{S}(h,Q) = h_{max}\mathbf{S}(Q) = h_{max}C\left(\frac{Q}{Q_{max}}\right)^a \left(\frac{Q_{max} - Q}{Q_{max}}\right)^b \tag{A1.11}$$

where h_{max} is the maximum hatching probability.

Typically, $h(Q)$ is a monotonically increasing function of Q, which means that hatching probability can be modeled by setting $b = 0$, giving

$$h(Q) = h_{max}\left(\frac{Q}{Q_{max}}\right)^a \qquad \text{(for } Q \le Q_{max}\text{; else } h(Q) = h_{max} \text{ for } Q > Q_{max}\text{)} \tag{A1.12}$$

The parameters h_{max}, Q_{max}, a and b of Eqns A1.11 and A1.12 may depend on species, gender and stage.

A1.4. Modeling survival and fecundity of the adult stage

In contrast to the immature stages, adults do not hatch to the next stage. Instead of hatching, adults may die due to aging. The risk of dying per time step is likely to increase as the biological age Q approaches the maximum age Q_{max}. Besides, ambient temperature may also have an effect on survival probability, especially at extreme temperatures. Thus, survival probability is modeled as a product of the ambient temperature (T) and an individual's biological age (Q). We model the likelihood that an individual will die during a time step in a way similar to hatching probability (Eqn A1.12), i.e. $d(Q) = d_{max}\left(\frac{Q}{Q_{max}}\right)^a = S(Q)$ because $d_{max} = 1$ when $Q = Q_{max}$. This gives the probability that an adult of age Q will survive the next time step when the temperature is T as

$$s(T,Q) = \mathbf{S}(s,T)\big(1 - \mathbf{S}(Q)\big) = s_{max}\mathbf{S}(T)(1 - \mathbf{S}(Q)) \tag{A1.13}$$

where $\mathbf{S}(T)$ and $\mathbf{S}(Q)$ are specific survival functions, modelled by the SANDY model.

Age-dependent oviposition rates of females may are modelled as a function of the ambient temperature (T) and the biological age (Q) of the females. Usually fecundity increases initially, probably due to maturation of eggs, and then declines as aging proceeds. We therefore model fecundity as

$$F(T,Q) = F_{\max}\mathbf{S}(T)\mathbf{S}(Q) \tag{A1.14}$$

where $F_{\max}$ is the maximum oviposition rate while $\mathbf{S}(T)$ and $\mathbf{S}(Q)$ are the specific functions modifying fecundity.

A1.5. Modeling intraspecific competition

We assume that high densities of host larvae in the manure reduce the per capita survival rate of larvae. Thus, the effect of intraspecific competition on survival is included in the model as a limiting factor given as

$$f(N) = \frac{1}{1+\psi\left(N/M\right)} \tag{A1.15}$$

where N is the number of larvae (i.e. $N = H_2$) and M is the volume of the manure while ψ is a constant expressing the strength of competition among larvae. For simplicity, it is assumed that ψ is the same for both gender and all age groups, though it seems likely that older larvae have a stronger impact on young larvae than vice versa. $f(N)$ approaches 1 when larval density approaches 0, and approaches 0 when $N/M \to \infty$.

A1.6. Modeling the interactions between parasitoids and hosts

Adult females of *S. cameroni* cause mortality on *S. calcitrans* by attacking host pupae. An attack may result in a successful oviposition, yielding a viable parasitoid offspring developing inside the puparium. However, not all attacks are successful as the pupa may die without yielding a parasitoid, either because the offspring dies before it is fully developed or because the host is used for host feeding. Attacks not resulting in a viable parasitoid are attributed to parasitoid induced mortality (PIM). Therefore, parasitoid fecundity can be modeled as $F = \varphi E$ where E is the number of attacks per time unit and φ is the proportion of attacks resulting in a successful oviposition, while 1-φ is the proportion dying due to PIM (Skovgård & Nachman 2015a). The fecundity of an adult female parasitoid of age x depends on the availability of suitable hosts and the parasitoid's ability to find an attack hosts. Besides, mutual interference between searching parasitoids may also affect the attack rate. The combined effect of temperature (T), biological age (Q), number of host pupae (N), and number of adult female parasitoids (P) on the attack rate is modeled as

$$E = E_{\max} f_1(T) f_2(Q) f_3(N) f_4(P) \tag{A1.16}$$

where $E_{\max}$ is the maximum rate of encounters with hosts. We model $f_1(T)$ and $f_2(Q)$ by means of the SANDY model, i.e. $f_1(T) = \mathbf{S}(T)$ and $f_2(Q) = \mathbf{S}(Q)$, while the effect of host density is modeled by means of the Weibull (1951) distribution given as

$$f_3(N) = 1 - e^{-\alpha(N_3/A)^{\beta}} \tag{A1.17}$$

where α and β are positive constant, which are assumed to be independent of parasitoid age and temperature (Nachman & Skovgård 2015a). Finally, the effect of parasitoid density (nutual interference) is modelled as

$$f_4(P) = \frac{1}{1+r(P/A)^q} \tag{A1.18}$$

where r and q are positive parameters (Nachman & Skovgård 2015b).

In the following, we will use x and y to denote the chronological age of host and parasitoid individuals, respectively. Thus, the total attack rate of the parasitoid population is modeled as

$$R = \sum_{y=0}^{\infty} E(y) P\left(2,2,y\right) \tag{A1.19}$$

where $E(y)$ is the attack rate per adult female (Eqn A1.16) and $P(2,2,y)$ is the number of adult females of age y.

Larsen (2006) carried out a series of experiments that showed that the attack rate of *S. cameroni* varies with the age of the host pupae. The probability that an encountered host is attack is modeled by means of the age-dependent function $\nu(x)$. Likewise, a proportion of the attacked hosts produce a viable parasitoid offspring modeled by the function $\varphi(x)$. Furthermore, when offered pupae of different ages, the parasitoids showed preference for parasitizing pupae of intermediate ages. The preference function is denoted $\pi(x)$. $\nu(x)$, $\varphi(x)$ and $\pi(x)$ are assumed to be independent of the gender of the fly pupae.

Host pupae of age x are assumed to be encountered in proportion to the age group's share of the total number of healthy pupae, i.e. $H_3(x)/H_3$. The total rate of attacks on pupae of gender g and age x is modeled as

$$Z'(g,x) = \nu(x)R\frac{\pi(x)H(g,3,x)}{\sum_{g=1}^{2}\sum_{x=0}^{\infty}\pi(x)H(g,3,x)} \tag{A1.20}$$

The number of attacked pupae of gender g and age x during Δt is found as

$$Z(g,x) = H_3(g,3,x)\left(1-e^{-Z'(g,x)\Delta t/H(g,3,x)}\right) \tag{A1.21}$$

and the number of ovipositions as

$$Z_p(g,x) = \varphi(x)Z(g,x) \tag{A1.22}$$

Survival probability of host pupae of age x to age x+1, i.e. Δt = 1 day, is modeled as a combination of temperature (Eqn A1.10) and attack risk, yielding

$$s(g,3,x) = \mathbf{S}(s,T)e^{-Z'(g,x)\Delta t/H(g,3,x)} \tag{A1.23}$$

A1.7. Modeling temperature variation in manure

Manure temperature in a given depth is assumed to depend on fermentation processes in the manure, ambient temperatures and the ability of manure to conduct heat. We consider manure as consisting of L vertical layers each of width ΔL = 1 cm. The change in temperature in layer i (corresponding to depth $L_i = i\Delta L$) is modeled as

$$\frac{dT_i}{dt} = K_i - c\left(T_i - T_{i-1}\right) - c(T_i - T_{i+1}) \tag{A1.24}$$

where T_i denotes the current temperature of layer i. K_i is the heat produced per time unit due to fermentation, and c is a constant expressing heat conductivity of manure. T_0 corresponds to the current air temperature and T_{L+1} to the floor temperature (T_{floor}). Until more information is available, K_i is regarded as a constant (i.e. $K_i = K$ for all i), though it is likely to depend on temperature and humidity in layer i. Likewise, c may also depend on the medium, e.g, heat may dissipate from manure to the air at a slower rate than the other way round.

A1.8. Modeling the vertical distribution of insects within the stable

Though stable fly larvae can actively move around in the manure to find suitable sites, we have for the time being too little information about the factors that influence such movements, because movements are constrained by many factors of which temperature, texture, water content and anoxia are likely to be the most important. Typically, manure becomes more compressed, wetter and with less oxygen at increasing depths, so even though the temperature may be favorable at greater depths, immature stable flies are likely to be found in the upper layers of the manure. For simplicity we assume that eggs are deposited in the upper L_1 cm of the manure and exposed to the average temperature in this layer $\left(T_{surface} = \frac{1}{L_1}\sum_{i=1}^{L_1} T_i\right)$, while larvae can occupy the manure down to L_2 cm. The average temperature in this layer is found as $T_{dung} = \frac{1}{L_2}\sum_{i=1}^{L_2} T_i$. We assumed that L_1 = 3 cm and L_2 = 8 cm, which means that larvae typically develop at higher temperatures than the eggs. When larvae are going to pupate, they tend to move to cooler areas closer to the surface, so we assume that pupae are exposed to $T_{surface}$. Here pupae are exposed to parasitism, so we assume that both immature and adult *S. cameroni* are exposed to $T_{surface}$. Adult stable flies are typically found sitting where temperature is higher

than in the air, especially during cold seasons, for instance by spending time on the cattle, the ceiling above cattle, sunny spots on the walls etc. We therefore assumed that adult stable flies are capable of finding places where the temperate is optimal (T_{opt}), defined as where their fitness is maximized. Fitness is defined as the product of their temperature dependent survival rate ($s(T)$) and fecundity rate ($F(T)$), so T_{opt} is found as the temperature that maximizes $s(T)F(T)$. Males are assumed to behave in the same way as females.

A1.9. Accumulation and removal of manure, and the effect on mortality

The cattle inside the stable are confined in C smaller areas called compartments. The area of compartment c is denoted A_c and the volume of manure in the compartment is denoted M_c. Hence, the depth of the manure in the compartment is found as $D_c = M_c/A_c$. The unit of depth is cm, which means that M_c has unit cm^3 and A_c has unit cm^2. Manure is assumed to weigh 1 g per cm^3.

Without removal of manure, the amount of manure in a compartment will gradually increase with time. To make the manure model simple, we assume that the amount of manure increases linearly with time until cleaning takes place, i.e.

$$M = M_0 + M_1\tau \tag{A1.25}$$

where M_0 is the amount left after the last removal in the compartment which took place τ days earlier and M_1 is the daily production of new manure (depends on the number and weight of animals etc in the compartment).

If M denotes the amount of manure just before removal and M_0 is the amount left after removal, the proportion removed is found as $p_r = (M - M_0)/M$ ($0 < p_r \le 1$).

Mortality caused by cleaning up the manure is assumed to be proportional to the effectiveness of the cleaning procedure. We assume that all immature stages are affected in the same proportion, so that survival of eggs, larvae, pupae of both species, as well as of the adult parasitoids, can be modeled by including the factor $f(p_r) = 1 - p_r$ in Eqn A1.10, whereas removal of manure is assumed not to inflict mortality onto the adult flies.

A1.10. Modeling dispersal of adult flies and parasitoids inside the stable.

Adult flies and parasitoids are able to move freely inside the stable and are therefore expected to spend most time in areas where conditions are most favorable in terms of reproduction. As we have no experimental data to model dispersal, we assume that the likelihood that a female of age x leaves compartment c during a time step is given as

$$p_c(x) = \frac{1}{1 + mF_c(x)} \tag{A1.26}$$

where $F_c(x)$ is the fecundity in compartment c during the time step and m a parameter determining the effect of fecundity on dispersal rate . For simplicity and due to lack of empirical data, we set $m = 1$.

We assume that there is no mortality during dispersal and that dispersing individuals arrive at a compartment proportionally to its area. Thus, if $N_c(x)$ denotes the number of females of age x in compartment c, the number of females arriving at compartment c during a time step is found as $\frac{A_c}{A}\sum_{c=1}^{C} p_c(x)N_c(x)$, where A_c is the area of compartment c and A the total area of all compartments. It means that some of the individuals leaving compartment c may return to the same compartment, i.e. with probability A_c/A. Finally, we assume that males disperse with the same rate as the females.

Dispersal from one compartment to another will enable the species to quickly recolonize compartments where manure has been removed recently.

A1.11. The effect of pesticide application

To reduce the number of fly larvae, the farmer may use a larvacide. Mortality is assumed to depend on the dose applied to the manure in compartment *c* and the time since the pesticide was applied. The dose (measured as concentration per volume manure) remaining in the manure after τ days is modeled as

$$D_c = D_0 e^{-\delta\tau} \tag{A1.27}$$

where D_0 is the dose applied and δ is a parameter expressing the speed at which the larvicide decays in the manure. If the compartment is sprayed at *n* occasions, the total dose remaining in the manure at day *t* is found as

$$D_c = \sum_{i=1}^{n} D_{0i} e^{-\delta\tau_i} \tag{A1.28}$$

whereD_{0i} is the dose of the *i*th treatment applied at day t-τ_i. τ_i is the time elapsed since the *i*th application.

It is assumed that the larvacide affects hatching from one larval stage to the next and from the the last larval stage to the pupal stage. As we have no data on the duration of the separate larval stages, we assume that the first larval stage ends when $Q = Q_{max}/3$, the next when $Q = 2Q_{max}/3$, and the last one before $Q = Q_{max}$. At each of these three occasions, daily mortality increases with a factor $f(D)$ given as

$$f(D) = \frac{\exp(\mu_0 + \mu_1 \log D)}{1 + \exp(\mu_0 + \mu_1 \log D)} \tag{A1.29}$$

where μ_0 and μ_1 are parameters expressing the lethality of the pesticide.

A1.12. Daily temperatures

Air temperature inside the stable (T_{air}) varies at a daily basis. T_{air} can either be obtained from measurements made in the stable or be derived via a calibration function from measurements made by the nearest weather station. Stable air temperature influences manure temperature, which is also updated at a daily basis by means of Eqn A1.24.

A1.13. The computer program

The model was converted into a computer program, called *The Fly Simulator,* written in *Delphi*® 2009 (see Appendix 3).

A1.14. Initializing the model

The model is initialized by entering the number of eggs, larvae, pupae and adults of the stable fly and the number of immature and adult parasitoids. The age distributions within each stage are calculated by assuming a stable age distribution defined by the survival function with a constant mortality rate, i.e

$$l_x = e^{-\mu(T)x} \tag{A1.30}$$

where $\mu(T)$ is the daily mortality rate at the temperature *T,* where *T* is the temperature at day 0. $\mu(T)$ is found as $\mu(T) = 1$-$s(T)$ where $s(T)$ is the daily survival rate found by means of Eqn A1.10.

The number of individuals in age class *x* of gender *g* and stage *s* is found from the equation

$$N(g,s) = \sum_{x=0}^{\infty} N(g,s,x) = \sum_{x=0}^{\infty} N(g,s,0) e^{-\mu(T)x} \tag{A1.31}$$

As $N(1,s)$ is found as $(1\text{-}p_f)N_s$ and $N(2,s)$ as $p_f N_s$, where N_s is the initial number of individuals belonging to stage *s*, we can obtain $N(g,s,0)$ as

$$N(g,s,0) = \frac{N(g,s)}{\sum_{x=0}^{\infty} e^{-\mu(T)x}} \tag{A1.32}$$

Finally, the initial number of individuals in age class x is calculated as

$$N(g,s,x) = N(g,s,0)e^{-\mu(T)x} = \frac{N(g,s)}{\sum_{x=0}^{\infty} e^{-\mu(T)x}} e^{-\mu(T)x} \tag{A1.33}$$

A1.15. List of mathematical symbols used in the model.

Symbol	Description
t	Time (in days)
Δt	Time step (one day in the model)
N	Population size at day t (sub-groups are indexed by gender, stage and age)
H	Number of hosts at day t (sub-groups are indexed by gender, stage and age)
P	Number of parasitoids at day t (sub-groups are indexed by gender, stage and age)
g	Index to mark gender (Male: $g = 1$; Female: $g = 2$)
s	Index to mark stage (Host: 1 = egg, 2 = larva, 3 = pupae, 4 = adult; Parasitoid: 1 = immature, 2 = adult)
S	Last stage in life cycle (Host: $S = 4$, Parasitoid: $S = 2$)
p_s	Probability that an individual remains in the same stage until next day
p_h	Probability that an individual hatches to the next stage during one day
p_f	Probability that an offspring is female
x	Chronological age (in days) of individuals (or specifically of hosts).
y	Chronological age (in days) of parasitoids
$F(\cdot)$	Daily fecundity of a female
$Q(x)$	Biological age of an individual with chronological age x
ΔQ	Increase in biological age during one day
T	Ambient temperature at day t (^{o}C)
$f(\bullet)$	Effect of a limiting factor
$s(\bullet)$	Survival function
$h(\bullet)$	Hatching function
M	Volume of manure
R	Total number of parasitoid attacks on hosts during one day
E	Instantaneous attack rate of a parasitoid female
ν	Probability that a host individual of a given age is attacked
φ	Probability that an attack on a host results in an oviposition
π	Relative preference of parasitoids for hosts of various ages
$Z(\bullet)$	Total attack rate directed towards a given age group of hosts
T_i	Manure temperature in depth i
C	Number of compartments in the stable
A_c	Area of compartment c
A	Total area of all compartments
M_c	Volume of manure in compartment c
M_1	Manure production per day in a compartment
p_r	Probability that an individual is killed due to removal of manure
D	Concentration of larvicide in manure
τ	Days since a treatment (removal of manure or spraying with larvicide)
p_c	Probability that a female emigrates from compartment c

TABLE A1.1. MATHEMATICAL SYMBOLS (EXCEPT PARAMETERS) USED IN THE MODEL.

Appendix 2: Estimation of model parameters

A2.1. *Stomoxys calcitrans*

A2.1.1. Survival and hatch rate of *S. calcitrans* eggs

Larsen & Thomsen (1941) give the duration of the egg stage at seven different temperatures (Table 8 p. 39 in Larsen & Thomsen). Duration varies from 240 hours at 13.5°C to ca 22 hours at 35°C. They also measured viability of eggs, i.e. the proportion of eggs that hatch to larvae. Another data set of egg viability is provided by Sutherland (1979; Table 2, p. 225).

In order to find the daily development, survival and hatching rates, we applied a spreadsheet in Excel in which each row gives, column by column, (*a*) the temperature (T), (*b*) the age (x) in hours, (*c*) the age in days (*d*), the time step Δt, (*e*) the increase in biological age at age x (ΔQ; Eqn A1.9), (*f*) the biological age ($Q(x)$; Eqn A1.5), (*g*) the number of eggs surviving at age x ($N(x)$), (*h*) the probability that an individual of age x survives during Δt ($s(x,T)$; Eqn A1.10),(*i*) the probability that it dies ($m(x,T) = 1\text{-}s(x,T)$), (*j*) the probability that it hatches ($h(x,T)$; Eqn A1.12), (*k*) the number of surviving individuals $N(x+1,T) = s(x,T)(1\text{-}h(x))N(x)$ to age $x+\Delta x$ (Eqn A1.1), (*l*) The number of lost eggs $N_l(x) = N(x)\text{-}N(x)$, (*m*) the number of died individuals $D(x) = (m(x,T)/(m(x,T)+h(x,T)))N_l(x,T)$, (*n*) the number of individuals hatching during Δt $H(x) = (h(x,T)/(m(x,T)+h(x,T)))N_l(x,T)$, and (*o*) the number of larvae at time t $L(x) = L(x\text{-}1)+H(x\text{-}1)$, (*p*) $H(x)x$.

As Δx in the Excel table was set to one hour, while the time unit of the model is a day, we converted survival probability per day to survival probability per hour as $s^{1/n}$, where s is the survival probability per day and n is the number of small time steps of length Δx constituting 1 day, i.e. $n = 1/\Delta x$. Likewise, the probability of hatching during Δx is found as $1\text{-}(1\text{-}h)^{1/n}$, where h is the hatching probability per day.

The probability that an egg survives to become a larva at temperature T is found as

$$s_{total}(T) = \frac{\sum_{x=0}^{\infty} H(x)}{N(0)} \tag{A2.1}$$

where $H(x)$ is the number of eggs that hatched at age x days.

The mean age of the larvae at hatching is found as

$$t_D(T) = \frac{\sum_{x=0}^{\infty} H(x)x}{\sum_{x=0}^{\infty} H(x)} \tag{A2.2}$$

In practice, we calculated the sums for $x \leq 500$ hours to ensure that $N(x) \rightarrow 0$. We used Solver in Excel to find the combination of parameters that simultaneously provided the best fit to the empirical values of egg stage duration given by Larsen & Thomsen (1941), and egg viability by Sutherland (1979) and Larsen & Thomsen (1941).

Figure A2.1 shows the observed and predicted duration of the egg stage at different temperatures. The parameters used for calculating the daily hatch rate are given in Table A2.3. The model predicts that no development takes place if the temperature is below 11.26°C.

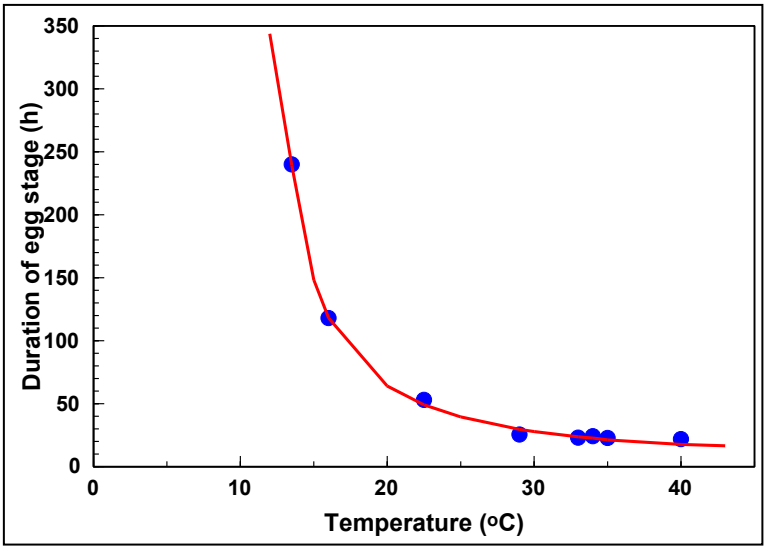

FIG. A2.1. OBSERVED (DOTS) AND PREDICTED (LINE) DURATION OF THE EGG STAGE OF STABLE FLIES. DATA FROM LARSEN & THOMSEN (1941). PREDICTIONS ARE BASED ON PARAMETER VALUES GIVEN IN TABLE A2.3.

Figure A2.2 shows the observed and predicted viability of stable fly eggs at different temperatures. The full line shows the predicted viability if only data from Larsen & Thomsen (1941) are used, implying that viability is 0% when the temperature is below T_{min} = 11.26°C. However, Sutherland found a viability of 84.9% at 10°C. We therefore combined the two data sets to estimate a common line for predicted viability (broken line). The parameters describing the relationship between temperature and daily survival rates are given in Table A2.3. The model predicts that the lower temperature threshold for egg survival (T_{min}) is 6.31°C.

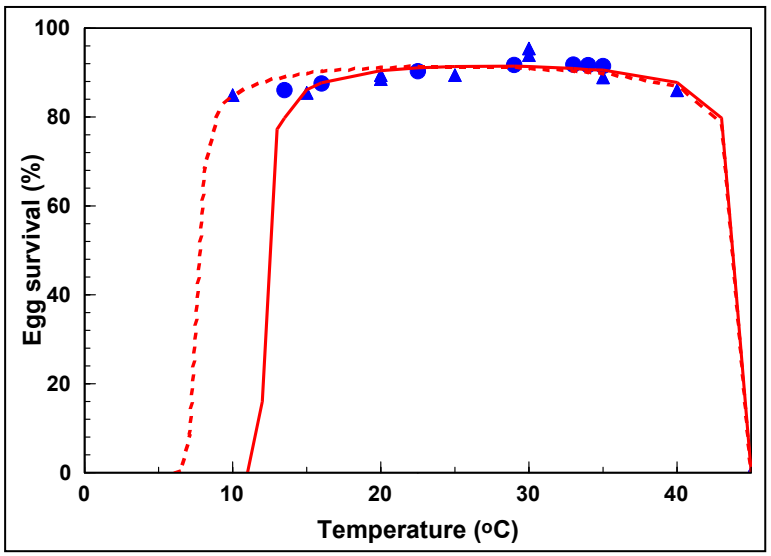

FIG. A2.2. OBSERVED (SYMBOLS) AND PREDICTED (LINES) VIABILITY OF STABLE FLY EGGS. DOTS SHOW DATA FROM LARSEN & THOMSEN (1941) AND TRIANGES SHOW DATA FRO SUTHERLAND (1979). THE FULL LINE SHOWS THE PREDICTED VIABILITY USING ONLY DATA FROM LARSEN & THOMSEN (1941) TO ESTIMATE THE PARAMETERS, WHILE THE BROKEN LINE IS BASED ON BOTH DATA SETS. PARAMETER VALUES ARE GIVEN IN TABLE A2.3.

A2.1.2. Survival and hatch rate of *S. calcitrans* larvae

Lysyk (1998) fitted the proportion of *S. calcitrans* larvae surviving the stage by the equation $y = 2.39 - 0.04T - 17.40/T$ (Table 1 in Lysyk). [Note that this equation does not correspond to the one shown in Fig. 2B in Lysyk (1998)], while Larsen & Thomsen (1941) give the duration of the larval stage in the temperature range from 13.4°C through 33°C. The parameters describing developmental, hatching and survival rates of larvae were estimated in the same way as for the eggs except that age steps (Δx) of 4 hours were used instead, corresponding to n = 6.

Figure A2.3 shows the observed and predicted duration of the larval stage at different temperatures while Fig. A2.4 shows the probability that a larva survives until the pupal stage.

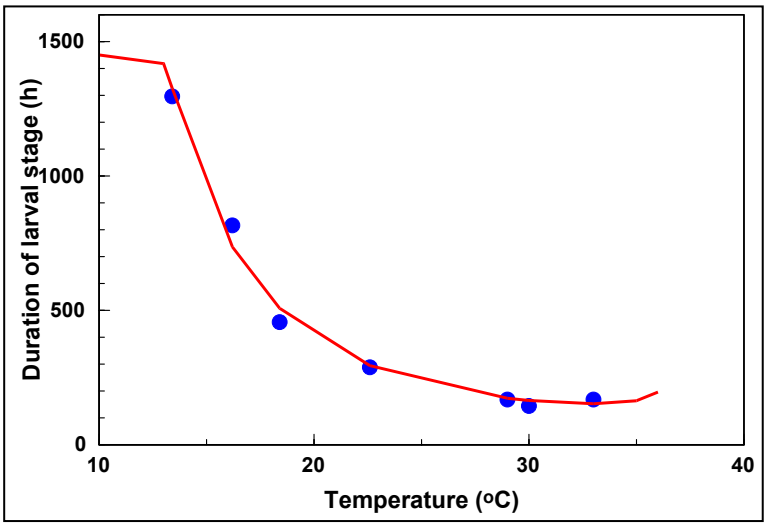

FIG A2.3. OBSERVED (DOTS) AND PREDICTED (LINE) DURATION OF THE LARVAL STAGE OF STABLE FLIES AT DIFFERENT TEMPERATURES. DATA FROM LARSEN & THOMSEN (1941). PREDICTIONS ARE BASED ON PARAMETER VALUES IN TABLE A2.3. LARVAE ARE PREDICTED TO DIE AT TEMPERATURES ABOVE 36.9°C.

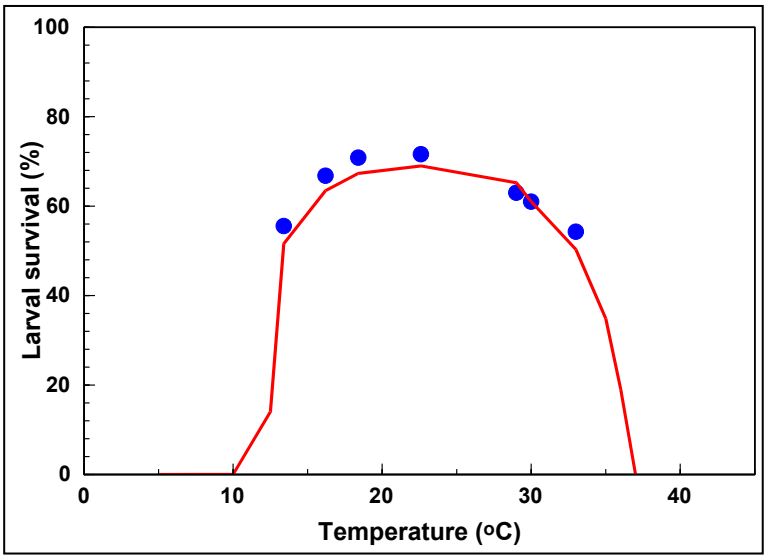

FIG. A2.4. OBSERVED (DOTS) AND PREDICTED (LINE) PROBABILITY THAT A LARVA SURVIVES THE LARVAL STAGE AT DIFFERENT TEMPERATURES. DATA FROM LYSYK (1998). PREDICTIONS ARE BASED ON PARAMETER VALUES IN TABLE A2.3.

A2.1.3. Survival and hatch rate of *S. calcitrans* pupae

We used the same procedure to estimate the parameters associated with the pupal stage as with the egg stage based on data in Sutherland (1979) and Larsen & Thomsen (1941). The parameters were obtained using age steps (Δx) of 4 h. Fig. A2.5 shows the observed and predicted probabilities of surviving the pupal stage, while Fig. A2.6 shows the observed and predicted duration of the stage.

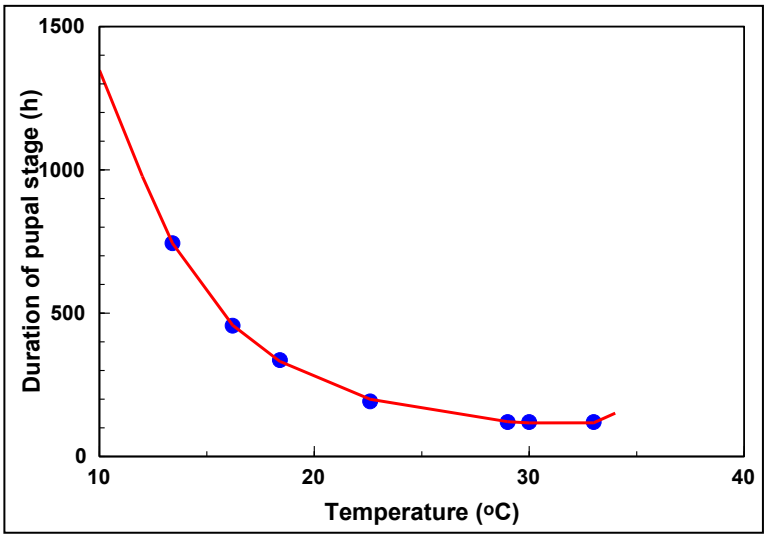

FIG. A2.5. OBSERVED (DOTS) AND PREDICTED (LINE) DURATION OF THE PUPAL STAGE OF STABLE FLIES AT DIFFERENT TEMPERATURES. DATA FROM LARSEN & THOMSEN (1941). PREDICTIONS ARE BASED ON PARAMETER VALUES IN TABLE A2.3. PUPAE ARE PREDICTED TO DIE AT TEMPERATURES ABOVE 34.4°C.

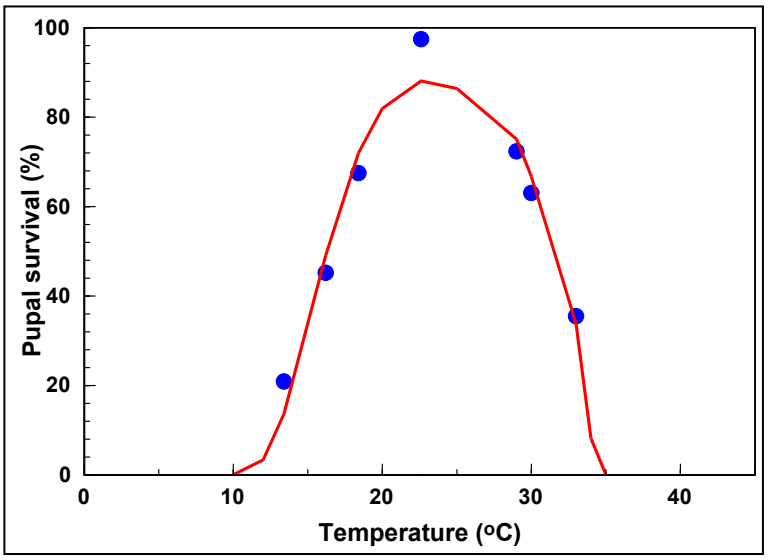

FIG. A2.6. OBSERVED (DOTS) AND PREDICTED (LINE) PROBABILITY THAT A PUPA SURVIVES THE ADULT STAGE AT DIFFERENT TEMPERATURES. DATA FROM LARSEN & THOMSEN (1941) AND SUTHERLAND (1979). PREDICTIONS ARE BASED ON PARAMETER VALUES IN TABLE A2.3.

A2.1.4. Survival and fecundity of adult *S. calcitrans*

To find the parameters of Eqns A1.13 and A1.14, allowing us to predict survival and fecundity rates of adult females at any temperature, we used data from Sutherland (1979) who gives MT_{50} values (mean time until 50% of the flies exposed to a given temperature have died), the pre-oviposition period, mortality during th pre-oviposition period, and the total number of eggs produced per ovipositing female. Values were obtained at 8 different temperatures ranging from 10°C to 45°C in steps of 5°C.

The mean survival time at temperature T (denoted $t_M(T)$) is found as

$$t_M(T) = \frac{\sum_{x=0}^{\infty} d(x,T)N(x)x}{\sum_{x=0}^{\infty} d(x,T)N(x)} \tag{A2.3}$$

where x is the age since hatching from pupa, and $d(x,T)$ is the probability that a female dies at age x (i.e. $d(x,T) = 1 - s(x,T)$). We calculated t_M values for a given temperature using Eqn A2.3. As s_{max}, T_{min}, T_{max}, a and b are unknown parameters, we used Solver in Excel to estimate these parameters as the values that minimize the sum of squared differences between t_M and the observed MT_{50} values for the temperatures used by Sutherland (1979) except 45°C, where no flies survived longer than 4 hours. It should, however, be noted, that t_M may be differ slightly from MT_{50} with respect to how they are defined, but for the purpose of estimating parameters this is considered a minor problem. Figure A2.9 shows the empirical values of MT_{50} and the predicted values of t_M. Sex ratio is set to 0.5 (Lysyk 1998).

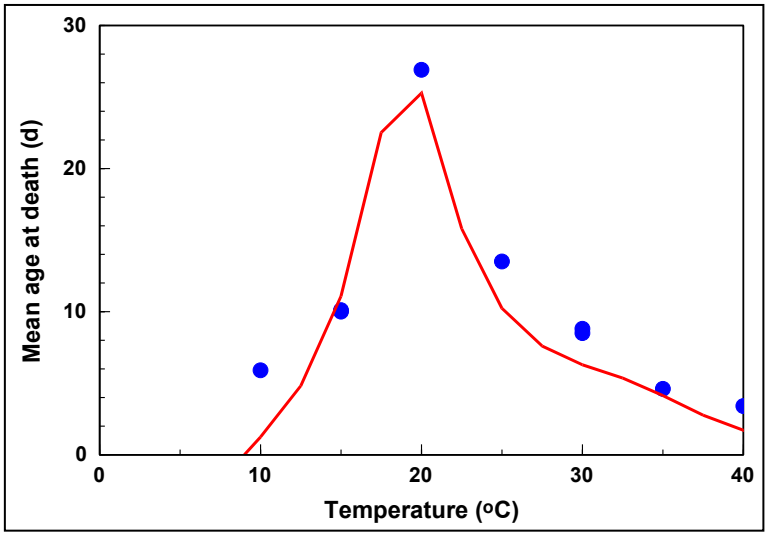

FIG. A2.7. THE OBSERVED (DOTS) AND PREDICTED (LINE) OF MT50 (TIME UNTIL 50% OF FEMALES IN A COHORT HAVE DIED) AND THE PREDICTED VALUES OF TM (THE MEAN SURVIVAL TIME OF FEMALES) AT 7 DIFFERENT TEMPERATURES. DATA FROM SUTHERLAND (1979). THE PREDICTED VALUES ARE BASED ON PARAMETER VALUES GIVEN IN TABLE A2.3.

The average total number of eggs produced by a female during her life time is computed as

$$F_{total}(T) = \frac{\sum_{x=0}^{\infty} N(x)F(x,T)}{N(0)} \qquad \text{(A2.4)}$$

The values of $F_{total}(T)$ were compared with the empirical values given by Sutherland (1979). These were calculated as F_{obs} = (100-P)E/100, where E is the average number of eggs produced per ovipositing female and P is the percentage of females dying during the pre-ovipositing period (Fig. A2.8).

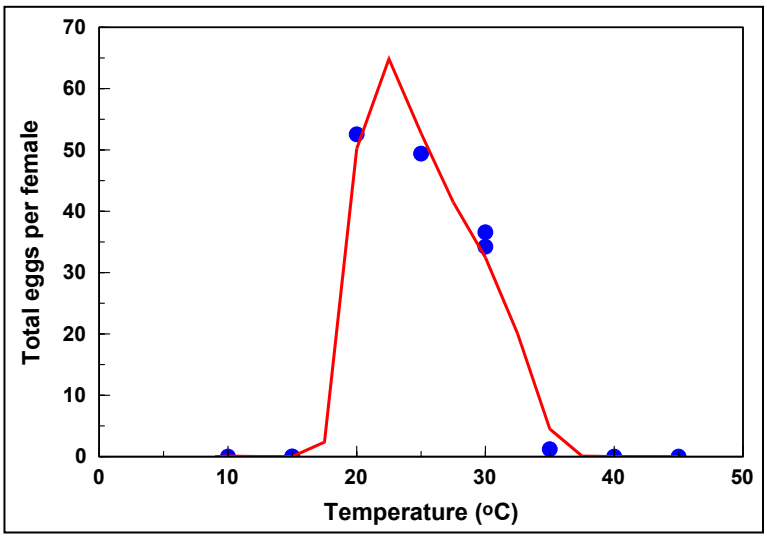

FIG. A2.8. OBSERVED (DOTS) AND PREDICTED (LINE) LIFE TIME FECUNDITY OF ADULT FEMALES OF STABLE FLIES. DATA FROM SUTHERLAND (1979). THE PREDICTED VALUES ARE BASED ON PARAMETER VALUES GIVEN IN TABLE A2.3.

Finally, Fig. A2.9 shows the fit of the model to life-table data of Salem et al. (2012) conducted at 25°C±2°C and based on 196 adult female stable flies. This is the only data set available where Eqns A1.13 and A1.14 can be fitted to the age-dependent survival and oviposition rates, which means that the parameters T_{min} and T_{max} cannot be estimated for the fecundity model. Instead we assumed that these parameters are the same as for survival. The parameter values of $\mathbf{S}(F,T)$ and $\mathbf{S}(Q)$ were estimated by means of Solver in Excel and are given in Table A2.3.

Sex ratio of the offspring is set to 50:50, i.e. $p_f = 0.5$. (Lysyk 1998)

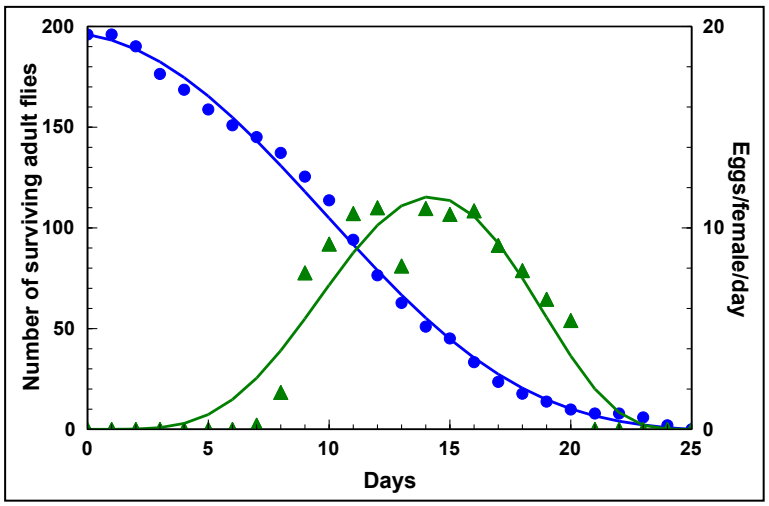

FIG. A2.9. OBSERVED SURVIVAL (DOTS) AND OVIPOSITION (TRIANGLES) RATES OF STABLE FLIES AT 25°C (SALEM ET AL. 2012). BLUE LINE IS THE PREDICTED SURVIVAL BASED ON EQN A1.13 AND GREEN LINE THE PRDICTED DAILY OVIPOSITION RATE BASED ON EQN A1.14. PARAMETER VALUES ARE GIVEN IN TABLE A2.3.

A2.2. *Spalangia cameroni*

A2.2.1. Survival and hatch rate of *S. cameroni* immatures

Development of the egg, larval, and pupal stages of *S. cameroni* takes place inside a fly puparium. Therefore, the duration and mortality of the individual stages could not be modeled separately, but were instead lumped into a single developmental stage called the "immature" stage.

In a series of experiments, pupae of *S. calcitrans* were exposed to abundant female *S. cameroni* to ensure that all available fly pupae were parasitized (Skovgård & Nachman subm.). Daily hatch rates were measured at 15, 20, 25, 30, and 35°C, and hatched individuals were sexed. Though males tend to hatch earlier than females, we pooled the two genders because the secondary sex ratio (i.e. the sex ratio when the eggs hatch) could not be estimated. Alternatively, the two genders can be modelled separately if it is assumed that the secondary sex ratio is the same as the tertiary sex ratio (i.e. the sex ratio when the adults emerge) (Skovgård & Nachman subm.). Furthermore, at each temperature two (three at 35°C) independent experiments were set up, each initiated with 100 newly parasitized fly pupae, but the two experiments were combined so estimates of hatch rate and survival were based on either 200 or 300 fly pupae.

The observed hatching probabilities were expressed as the likelihood that a host pupa resulted in an adult parasitoid and was estimated by dividing the number of emerging adult parasitoids by the initial number of pupae. Non-hatched pupae were subsequently dissected to find the cause of mortality (Skovgård & Nachman subm.), but for our analysis we simply regarded the pupae as black boxes and pooled all mortality causes into a common daily mortality rate. Thus, mortality also included pupae that were attacked but without producing a viable parasitoid either because the egg did not develop or because the pupa was used for host feeding (parasitoid induced mortality or PIM).

The predicted numbers of hatching *Spalangia* were found by simultaneously fitting Eqn A1.9 (to account for developmental rate), Eqn A1.10 (to account for survival) and Eqn A1.12 (to account for hatching) to the observed cumulated number of emerging adults (Fig. A2.10). Figure A2.11 shows the observed and predicted total hatch rate and Fig. A2.12 shows the observed and predicted mean duration of the immature stage. To limit the number of parameters to

be estimated, it is assumed that temperature thresholds T_{min} and T_{max} are the same for both the aging process and survival rates. Females constituted 56.2 % of the hatched individuals (SE = 2.1%).

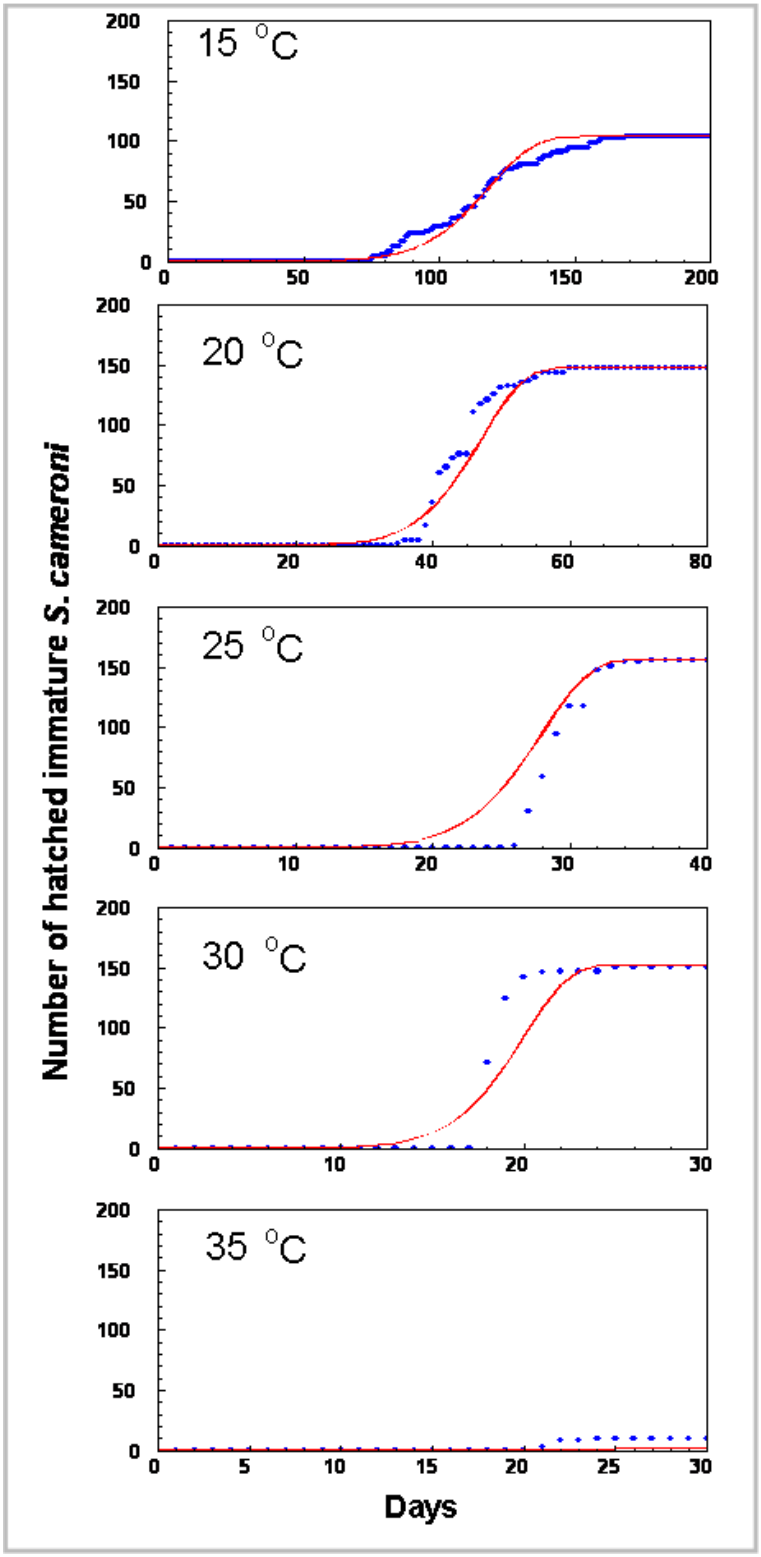

FIG. A2. 10. OBSERVED (DOTS) AND PREDICTED (LINES) CUMULATED NUMBER OF HATCHED IMMATURE *S. CAMERONI* FROM PUPAE OF *M. DOMESTICA* AT FIVE DIFFERENT TEMPERATURES. DATA FROM SKOVGÅRD & NACHMAN (SUBM). PREDICTED VALUES ARE BASED ON PARAMETER VALUES IN TABLE A2.4.

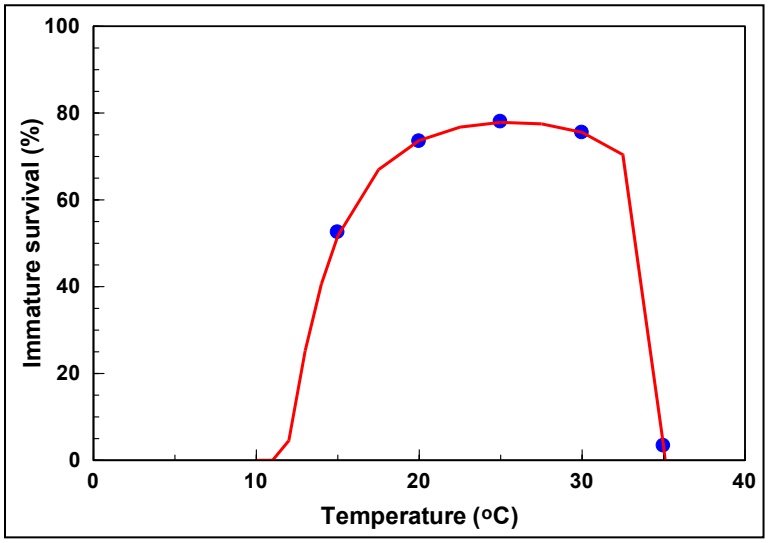

FIG A2.11. OBSERVED (DOTS) AND PREDICTED (LINE) SURVIVAL RATE OF IMMATURE *S. CAMERONI* AT DIFFERENT TEMPERATURES. DATA FROM SKOVGÅRD & NACHMAN (SUBM.). PREDICTED VALUES ARE BASED ON PARAMETER VALUES GIVEN IN TABLE A2.4 .

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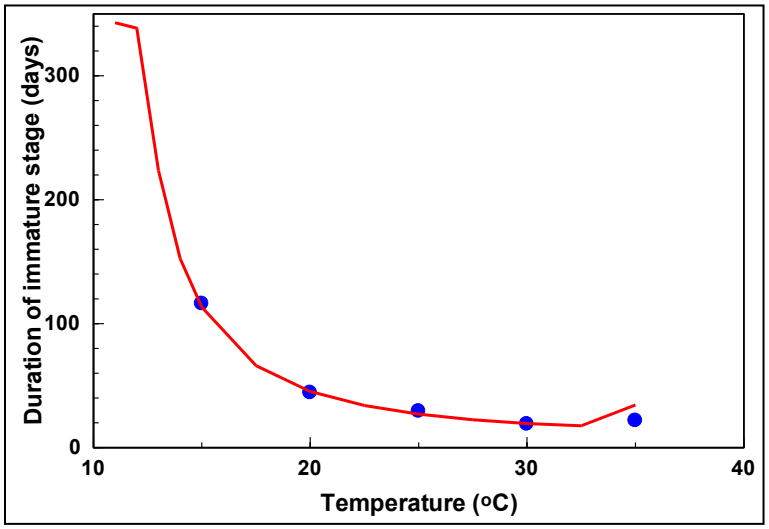

FIG A2.12. OBSERVED (DOTS) AND PREDICTED (LINE) DEVELOPMENTAL TIME OF IMMATURE *S. CAMERONI* FROM PUPAE OF *M. DOMESTICA* AT DIFFERENT TEMPERATURES. DATA FROM SKOVGÅRD AND NACHM (SUBM.). PREDICTED VALUES ARE BASED ON PARAMETER VALUES GIVEN IN TABLE A2.4.

A2.2.2. Survival and oviposition rates of *S. cameroni* females

Daily survival and fecundity rates of *S. cameroni* adult females were obtained from experiments conducted at five different temperatures (15, 20, 25, 30, 35°C) (Skovgård & Nachman subm.). Cohorts of newly emerged female parasitoids (25, 25, 22, 21, and 25 females, respectively) were followed individually until all individuals in the cohort had died. Each female was placed in a 7.1 cm² petri dish together with 25 young (2-3 d) pupae of house fly (*Musca domestica*). The pupae were exposed to the parasitoid for one day and then replaced by new pupae. The removed pupae were incubated until emergence of adult parasitoids. These pupae were scored as ovipositions. The number of attacked pupae was calculated as the difference between the number of pupae offered and the number of pupae producing an adult fly, while the difference between attacked pupae and ovipositions was categorized as PIM. Figure A12.13 shows the age- and temperature-dependent survival and attack rates. Adult daily survival probability was estimated by simultaneously fitting Eqn A1.9 (to account for the developmental rate) and Eqn A1.13 (to account for mortality) to survival data (see Section A2.1.1).

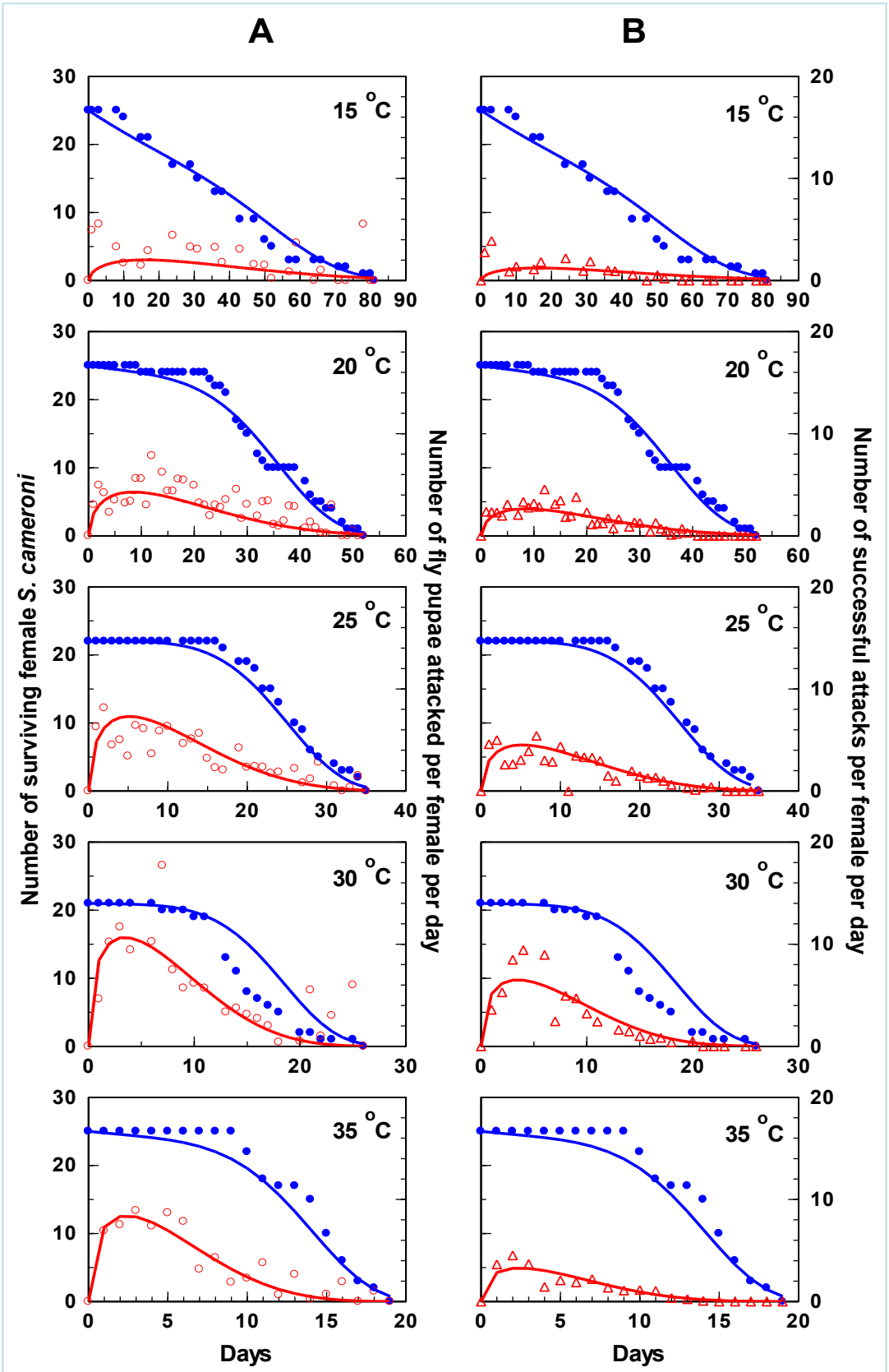

FIG. A2.13. OBSERVED (SYMBOLS) AND PREDICTED (LINES) SURVIVAL AND PER CAPITA ATTACK RATES OF *S. CAMERONI*. A: ALL ATTACKS. B: SUCCESSFUL ATTACKS (I.E. NUMBER OF ADULT PARASITOIDS EMERGING FROM ATTACKED PUPAE OF *M. DOMESTICAE*). BLUE DOTS AND LINES: SURVIVAL. RED DOTS AND LINES: ATTACKS. RED TRIANGLES AND LINES: SUCCESSFUL ATTACKS. DATA FROM SKOVGÅRD & NACHMAN (SUBM.). THE PREDICTED VALUES ARE BASED ON PARAMETERS IN TABLE A2.4.

Oviposition rate depends on the attack rate of the female parasitoids and the proportion of attacks (φ) that lead to a parasitoid offspring (successful attacks). It is modeled as

$$F(T,Q) = \varphi(T,Q)E(T,Q) \tag{A2.5}$$

where $E(T,Q)$ is the attack rate of a female parasitoid of biological age Q at temperature T. $E(T,Q)$ is modelled by means of Eqn. A1.16, but because only a single parasitoid was used in each experiment, mutual interference could be ignored, i.e. $f_4(P) = 1$, so Eqn A1.16 reduces to

$$E = E'_{\max} f_1(T) f_2(Q) \tag{A2.6}$$

where $E'_{\max} = E_{\max} f_3(N)$ is the maximum attack rate at the density used in the experiments, i.e. 25/7.1 = 3.52 pupae/cm². $f_1(T)$ is the effect of temperature and $f_2(Q)$ is the effect of age on attack rate. Both are modelled by the Sandy model, i.e. $f_1(T) = \mathbf{S}(T)$ and $f_2(Q) = \mathbf{S}(Q)$.

$\varphi(T,Q)$ is the success ratio at temperature T and biological age Q . It is modeled by the SANDY model as

$$\varphi(T,Q) = \mathbf{S}(\varphi,T)S(Q) = \varphi_{\max}\mathbf{S}(T)\mathbf{S}(Q) \tag{A2.7}$$

where $\varphi_{\max}$ is the maximum success ratio. To limit the number of estimated parameters it is assumed that $T_{\min}$ and $T_{\max}$ are the same for survival rates as for the aging process. Likewise, it is assumed that $Q_{\max}$ is the same for survival rate as for the attack and oviposition rates. The predicted values in Fig. A2.13 were obtained by fitting the models to the experimental data using the parameters values given in Table A2.4. However, the estimated parameter values associated with temperature effects on attack rate and success ratio were not used in the simulation model, because the functional response experiments described in Section A2.2.3 also allows for estimating the effect of host density on the attack rate. It means that $E_{\max}$ and $f_3(N)$ can be estimated separately instead of lumping them into $E'_{\max}$.

A2.2.3. Effect of temperature and host burial depth on the functional response

Skovgård & Nachman (2015a) carried out a series of experiments where young *S. cameroni* females were exposed to 2, 4, 8, 16, 32 and 64 pupae of *S. calcitrans* placed at three different depths (0, 8 and 16 cm) in the substrate consisting of unstained whole wheat grains. The experiments were conducted at five constant temperatures (15, 20, 25, 30 and 35°C) and each combination was replicated six times. The surface area of the substrate (A) was 19.64 cm² and an experiment lasted 24 h.

The results show that host density and temperature have a highly significant effect on the proportion of the offered fly pupae to be attacked ($P < 0.0001$; logistic regression), whereas the effect of depth was insignificant (Skovgård & Nachman 2015a).

The instantaneous rate of encounters with hosts at temperature T ($E(T)$) is modelled by means of Eqn. A1.16, which reduces to

$$E(T) = E_{\max} f_1(T) f_3(N) \tag{A2.8}$$

because $f_2(Q)$ of young parasitoid females is set to 1 and $f_4(P) = 1$ because only a single parasitoid was present. $f_1(T)$ is modelled by means of the Sandy model, i.e. $f_3(T) = \mathbf{S}(T)$ and $f_3(N)$ with Ivlev's (1961) model (Eqn. A1.17). If Eqn. A2.8 is fitted to the functional response at each temperature separately, Eqn. A2.8 can be simplified as

$$E(T) = E_m(T) f_3(N) \tag{A2.9}$$

where $E_m(T) = E_{\max} f_1(T)$ is the maximum attack rate at temperature T.

The finite rate of attack during the 24h at temperature T is found as

$$N_a(T) = N\left(1 - e^{-(E(T)/N)\Delta t}\right) \tag{A2.10}$$

where N is the number of available pupae.

Figure A2.14 (A) shows the fit of Eqn. A2.10 to the number of attacked hosts. α and β were found to be independent of temperature and since β was close to unity, this parameter was set to 1, indicating that the functional response of *S. cameroni* is a type II. $E_m(T)$ was estimated for each temperature separately and fitted by the SANDY model (Fig. A2.15)

The rate of successful attacks (ovipositions) is found as

$$F(T) = \varphi(T)N_a(T) \tag{A2.11}$$

where $\varphi(T)$ denotes the proportion of attacked hosts that result in a viable offspring, while 1-$\varphi(T)$ is the proportion of parasitoid induced mortality (PIM). Figure A2.14 (B) shows the observed and predicted oviposition rates, while Fig. A2.16 shows the fit of the SANDY model to the temperature-dependence of φ.

The sex ratio of the offspring (expressed as proportion of females p_f) was found to be independent of temperature and depth (Skovgård & Nachman 2015a). The overall value of p_f was found to be 0.707 (95% CL: 0.677-0.738).

The value of E_{max} was found to be higher than the one found from the life-table data (20.230 vs 18.3595 attack/day; Table A2.4). However, it has to be noted that E_{max} in Skovgård & Nachman (subm.) corresponds to the maximum attack rate against a fixed density of hosts (3.521 pupae/cm^2), whereas E_{max} in Skovgård & Nachman (2015a) represents the maximum attack rate if host density approaches infinity. Furthermore, Skovgård & Nachman (subm.) used house flies while Skovgård & Nachman (2015a) used stable flies as host. As the simulation model focuses on stable flies as hosts, we apply the parameter values obtained from the latter study to model the effect of temperature and host density on the attack and oviposition rates of *S. cameroni* (Table A2.4).

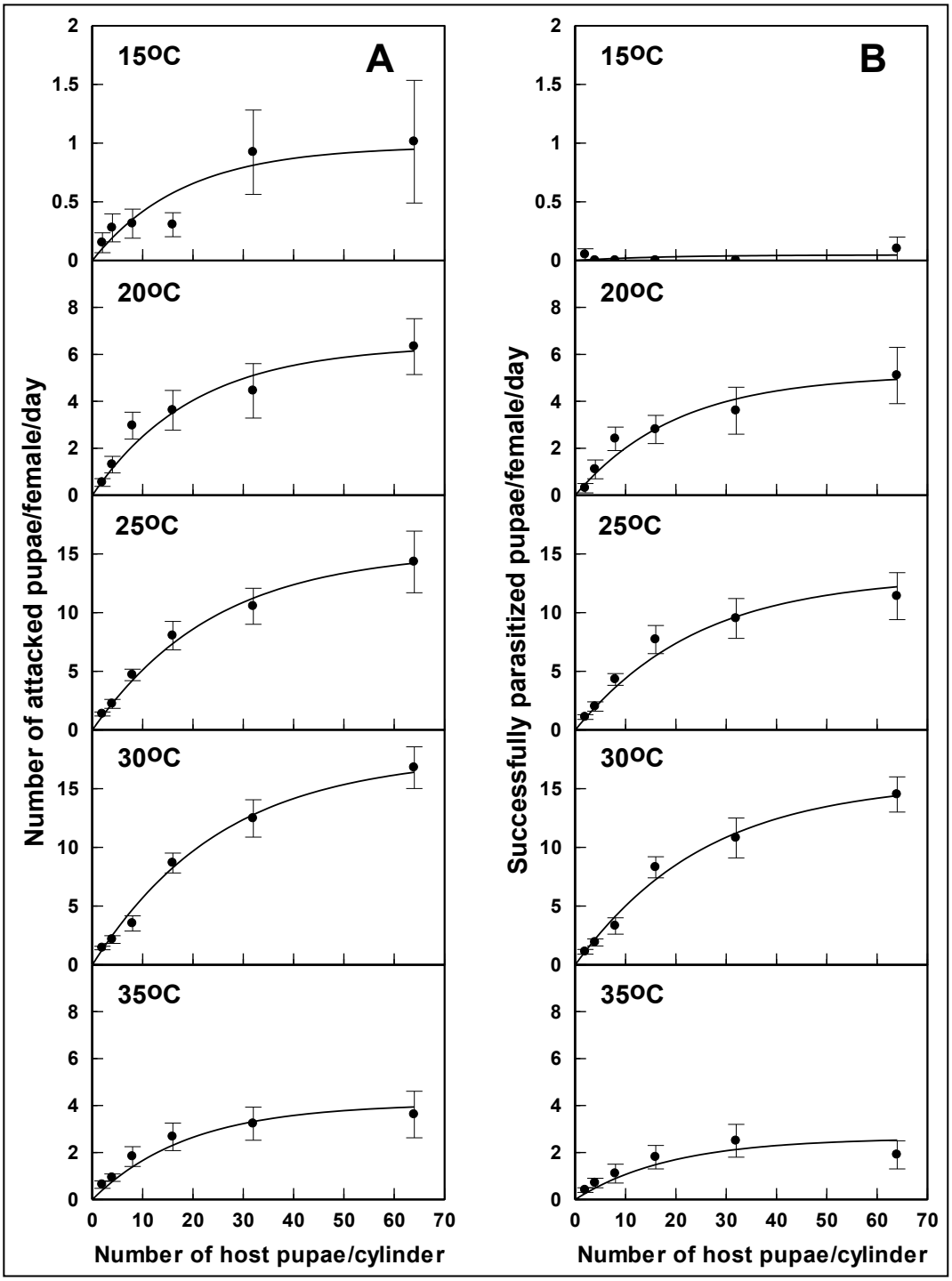

FIG. A2.14. FUNCTIONAL RESPONSES OF ADULT FEMALE *S. CAMERONI* ATTACKING PUPAE OF STABLE FLIES (*S. CALCITRANS*) AT FIVE DIFFERENT TEMPERATURES. DATA FROM SKOVGÅRD & NACHMAN (2015A). POINTS REPRESENT THE OBSERVED NUMBER OF ATTACKED PUPAE (PANEL A) AND THE OBSERVED NUMBER OF SUCCESSFUL OVIPOSITIONS (PANEL B) AFTER 24 HOURS (±SE). EACH POINT IS BASED ON THE AVERAGE OF 16-18 OBSERVATIONS (OBTAINED AT 3 DEPTHS AND 6 REPLICATES PER DEPTHS). THE LINES SHOW THE PREDICTED VALUES BASED ON EQN A2.10 (PANEL A) AND EQN A2.11 (PANEL B) AND WITH PARAMETER VALUES GIVEN IN TABLE A2.4.

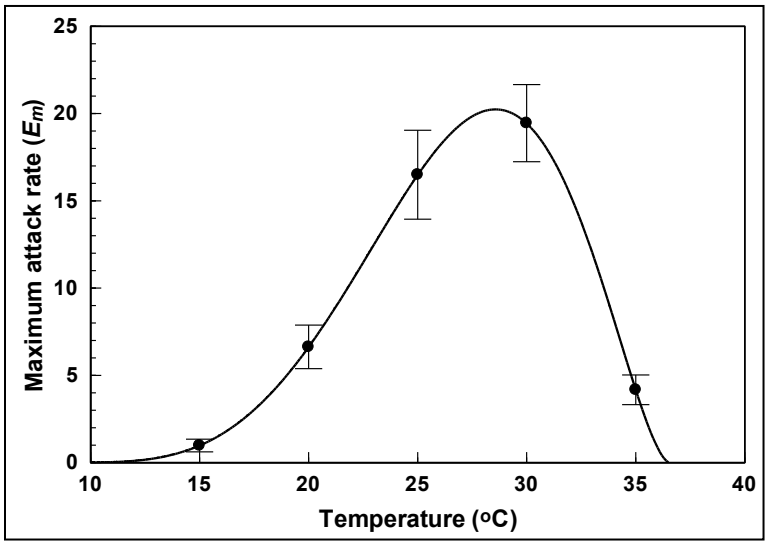

FIG. A2.15. TEMPERATURE DEPENDENCE OF THE MAXIMUM ATTACK RATE (E_M). DATA FROM SKOVGÅRD & NACHMAN (2015A). DOTS REPRESENT THE EMPIRICAL VALUES (±SE) AND THE LINE THE FIT OF THE SANDY MODEL TO DATA WITH PARAMETER VALUES GIVEN IN TABLE A2.4.

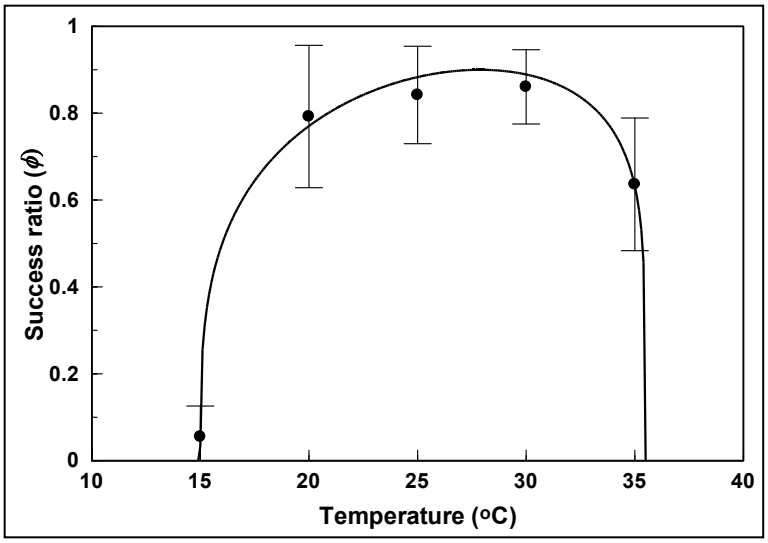

FIG. A2.16. TEMPERATURE-DEPENDENCE OF THE FRACTION OF ATTACKS (Φ) CARRIED OUT BY ADULT FEMALE *S. CAMERONI* LEADING TO SUCCESSFUL OVIPOSITIONS. DATA FROM SKOVGÅRD & NACHMAN (2015A). DOTS REPRESENT THE EMPIRICAL VALUES (±SE) AND THE LINE THE FIT OF THE SANDY MODEL TO DATA WITH PARAMETER VALUES GIVEN IN TABLE A2.4.

A2.2.4. Effect of mutual interference on attack and oviposition rates

Based on the experiments by Skovgård & Nachman (2015b) it was found that the per capita rate attack rate declines with parasitoid density (Fig. A2.17). The predicted effect of mutual interference on attack rate is described by Eqn. A1.18 (Fig.A2.18).

Skovgård & Nachman (2015b) also found that the proportion of attacks leading to a successful parasitation (φ) also declines with parasitoid density. The effect of parasitoid density on φ at 25°C is also modelled by means of Eqn A2.18, but with other parameter values than those used for modelling attack rates (Table A2.4). The relationship between parasitoid density and successful attacks is shown in Fig. A2.19.

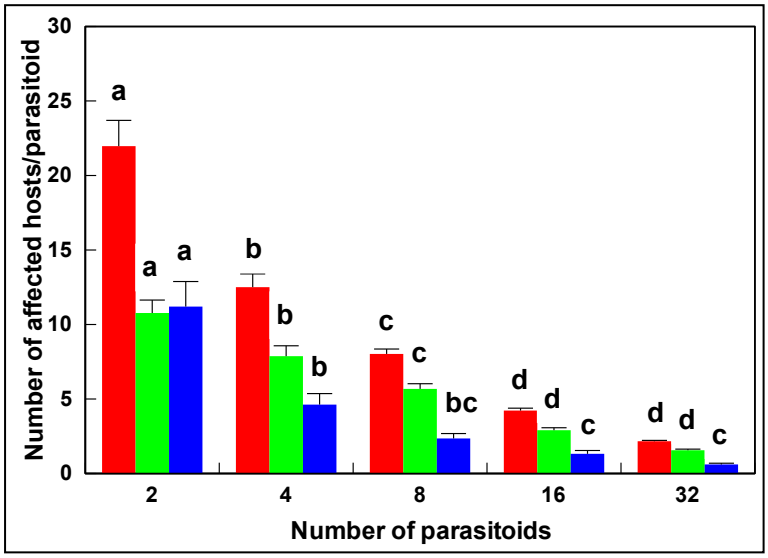

FIG. A2.17. THE EFFECT OF PARASITOID DENSITY ON THE PER CAPITA ATTACK RATE (RED BARS), OVIPOSITION RATE (GREEN BARS), AND PIM (BLUE BARS) OF *S. CAMERONI* FEMALES. DATA FROM SKOVGÅRD & NACHMAN (2015B). VERTICAL BARS SHOW STANDARD ERRORS. BARS WITH THE SAME COLOUR ARE SIGNIFICANTLY DIFFERENT ($P < 0.05$) IF THE LETTERS ARE DIFFERENT. THE AREA OF THE EXPERIMENTAL ARENA IS 25.52 CM².

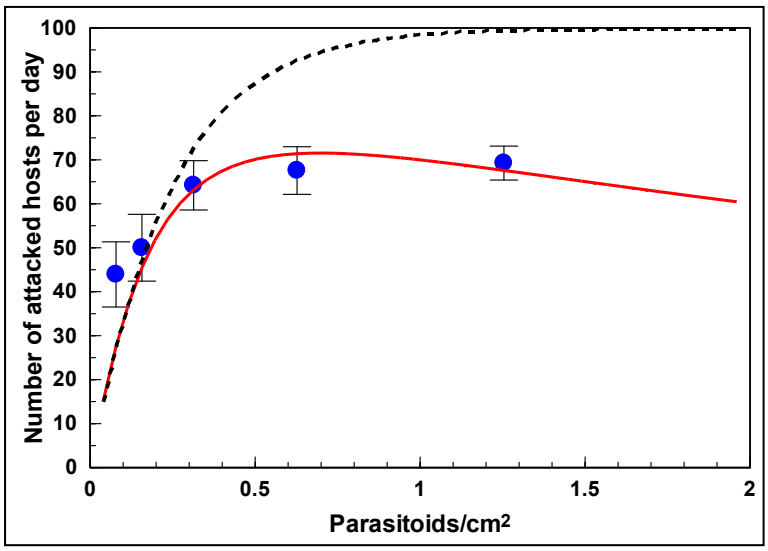

FIG. A2.18. EFFECT OF PARASITOID DENSITY ON THE OBSERVED (DOTS ± 95% CONFIDENCE INTERVALS) AND PREDICTED ATTACK RATESOF *S. CAMERONI* ADULT FEMALES. DATA FROM SKOVGÅRD & NACHMAN (2015B). THE BROKEN LINE SHOWS THE PREDICTED ATTACK RATE IF MUTUAL INTERFERENCE DOES NOT TAKE PLACE WHILE THE RED LINE IS THE PREDICTED VALUES BASED ON EQN A1.16 INCORPORATING THE EFFECT OF MUTUAL INTERFERENCE (EQN A1.18), USING THE PARAMETER VALUES IN TABLES A2.4 AND A2.5.

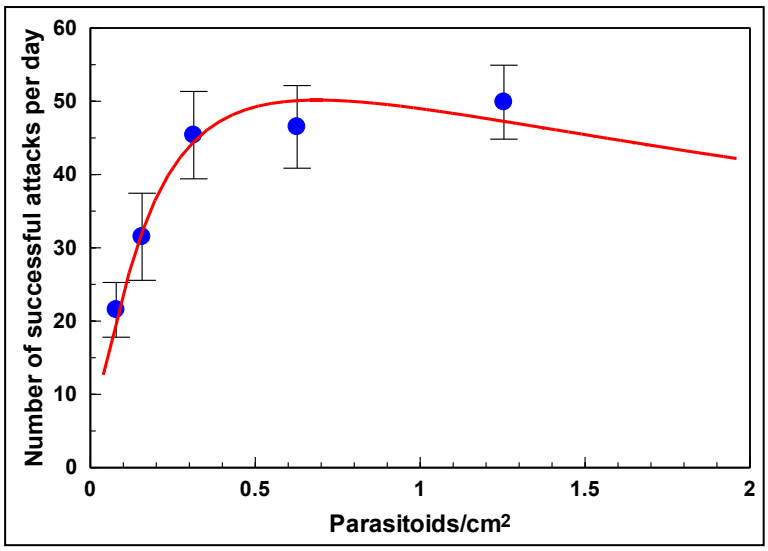

FIG. A2.19. EFFECT OF PARASITOID DENSITY ON THE OBSERVED (DOTS ± 95% CONFIDENCE INTERVALS) AND PREDICTED OVIPOSITION RATES OF *S. CAMERONI* ADULT FEMALES. DATA FROM SKOVGÅRD & NACHMAN (2015B). THE PREDICTED VALUES ARE BASED ON EQN A2.11 BY MODELLING THE EFFECT OF MUTUAL INTERFERENCE ON BOTH THE ATTACK RATE AND THE SUCCESS RATIO BY MEANS OF EQN A1.18 USING THE PARAMETER VALUES IN TABLE A2.4 AND A2.5.

A2.2.5. Effect of host age on attack and oviposition rates of *S. cameroni*

Larsen (2006) conducted two series of experiments where he examined *S. cameroni*'s ability to parasitize *S. calcitrans* pupae of various ages. In experiment 1, young female parasitoids were shortly after being mated introduced singly to 5 host pupae of the same age. Seven age groups were used, namely 0-1,1-2, 2-3, 3-4, 4-5, 5-6, and 6-7 days old pupae. In experiment 2, the parasitoid was simultaneously introduced to 14 host pupae, 2 pupae from each of the seven age groups used in experiment 1. The temperature was 25°C and the duration of an individual experiment was 2 hours. Each experiment was replicated 10 times.

In experiment 1, 3-4 days old pupae were the age group in which most pupae were attacked, namely 4.57 of which 3.45 resulted in an oviposition, i.e. $F(x_m) = \varphi(x_m)N_a(x_m)$, where $F(x)$ and $N_a(x)$ denote the number of parasitized and attacked pupae, respectively, when host age is x days and x_m is the age at which $F(x)$ has a maximum. Attack rate against pupae of age x ($N_a(x)$) is modeled as

$$N_a(x) = \nu(x)N_a(x_m) \tag{A2.12}$$

where $\nu(x_m) = \nu_{max} = 1$. Therefore, the number of successfully parasitized pupae of age x is modeled as

$$F(x) = \varphi(x)\nu(x)N_a(x_m) \tag{A2.13}$$

Instead of modeling $\nu(x)$ and $\varphi(x)$ as functions of chronological age, age was converted to the biological age of an individual in age group x, i.e. the mid-point of the age interval $[x; x+1[$, found as $Q = Q(x) = (x+0.5)\Delta Q(T)$, where $\Delta Q(T)$ is calculated from Eqn. A1.9 at $T = 25$°C, using the parameters for *Stomoxys* pupae. The relationships between Q and $\nu(Q)$ and $\varphi(Q)$ were modeled by the SANDY model as $\mathbf{S}(\nu,Q)$ and $\mathbf{S}(\varphi,Q)$ where φ_{max} is the maximum value of φ measured at 25°C.

Figure A2.20 shows that young pupae have a lower risk of being attacked than older pupae have. The proportion of attacked hosts that were used for oviposition seems to be independent of host age and around 80% (Fig. A2.21).

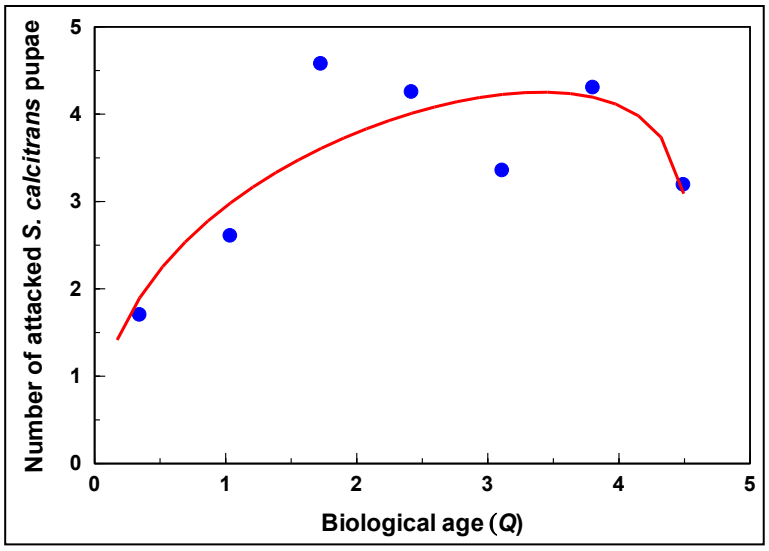

FIG. A2.20. OBSERVED (DOTS) AND PREDICTED (LINE) ATTACK RATE OF *S. CAMERONI* FEMALES AGAINST *S. CALCITRANS* PUPAE OF SEVEN DIFFERENT AGE GROUPS. DATA FROM LARSEN (2006). THE PREDICTED VALUES ARE BASED ON EQN. A2.12, BUT USING BIOLOGICAL AGE (Q) INSTEAD OF CHRONOLOGICAL AGE (X). PARAMETERS ARE GIVEN IN TABLE A2.4.

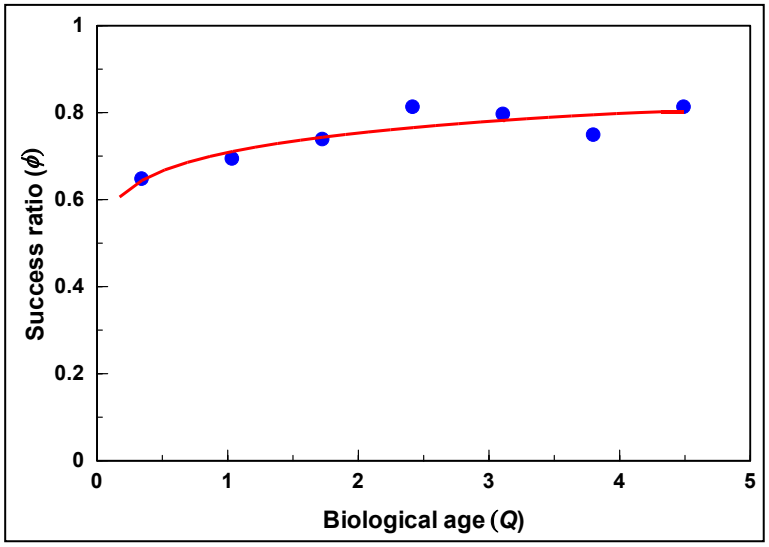

FIG. A2.21. OBSERVED (DOTS) AND PREDICTED (LINE) PROPORTION OF ATTACKS THAT WERE SUCCESSFUL (PRODUCED VIABLE OFFSPRING) WHEN FEMALES OF *S. CAMERONI* WERE OFFERED *S. CALCITRANS* PUPAE OF DIFFERENT AGE (FIG. A2.20). DATA FROM LARSEN (2006). THE PREDICTED VALUES ARE BASED ON EQN. A2.13, BUT BIOLOGICAL AGE (Q) IS USED INSTEAD OF CHRONOLOGICAL AGE (X). PARAMETERS ARE GIVEN IN TABLE A2.4.

In experiment 2, pupae of different ages were offered simultaneously to the parasitoids. The number of attacked pupae averaged over the 10 replicates is 3.49. Figure A2.22 shows the expected number of pupae attacked in each age group calculated from Eqn. A2.12 as $N_a(x) = \nu(x) N_a(x_m)$ and where $\nu(x)$ is the function shown in Fig. A2.21. $N_a(x_m)$ is unknown but can be found from $N_a(x_m) \sum_{x=0}^{6} \nu(x) = 3.49$. As seen from Fig. A2.22, the agreement between observations and predictions is acceptable.

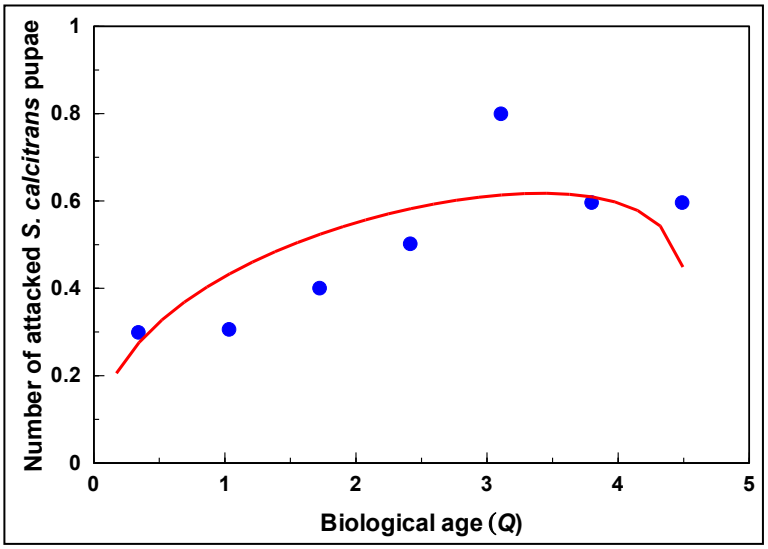

FIG. A2.22. OBSERVED (DOTS) AND PREDICTED (LINE) ATTACK RATE OF *S. CAMERONI* FEMALES AGAINST *S. CALCITRANS* PUPAE OF SEVEN DIFFERENT AGE GROUPS. TWO PUPAE OF EACH AGE GROUP WERE OFFERED SIMULTANOUSLY TO THE PARASITOID. DATA FROM LARSEN (2006). THE PREDICTED VALUES ARE BASED ON EQN. A2.12, BUT USING BIOLOGICAL AGE (Q) INSTEAD OF CHRONOLOGICAL AGE (X). PARAMETERS ARE GIVEN IN TABLE A2.4.

The number of host pupae of age x that yielded a parasitoid offspring is denoted $F(x)$. $\varphi(x)$ is the proportion of attacked hosts that are used for oviposition, i.e. $\varphi(x) = F(x)/N_a(x)$. Figure A2.23 shows the observed values of $\varphi(x)$ together with the predicted values obtained from Eqn A2.13 (red line).

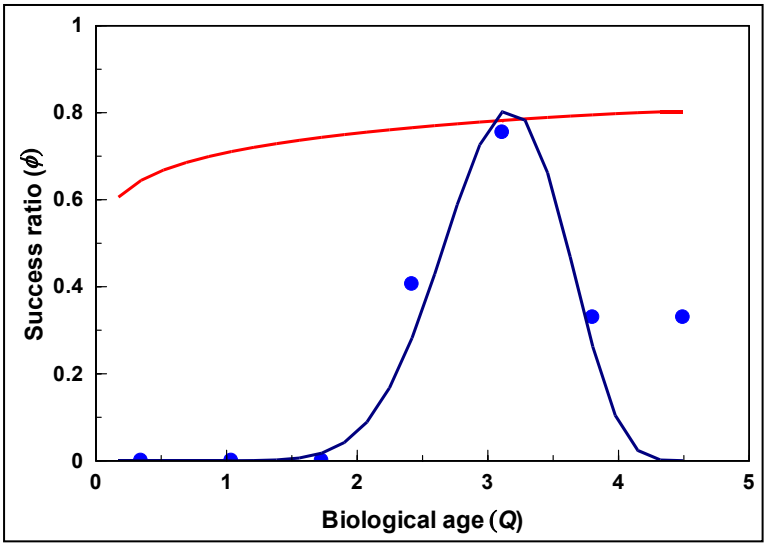

FIG. A2.23. EFFECT OF HOST AGE ON THE PROPORTION OF ATTACKS THAT ARE SUCCESSFUL WHEN HOST PUPAE OF *S. CALCITRANS* OF 7 DIFFERENT AGE GROUPS ARE SIMULTANEOUSLY OFFERED TO FEMALES OF *S.* (2 PUPAE PER AGE GROUP). DATA FROM LARSEN (2006). THE RED LINE SHOWS THE PREDICTED RELATIONSHIP BASED ON THE EXPERIMENTS WHERE ONLY ONE AGE GROUP IS USED (EQN. A2.13). THE BLACK LINE SHOWS THE PREDICTED VALUES WHEN THE PREFERENCE FOR CHOOSING PUPAE OF INTERMEDIATE AGE FOR OVIPOSITING IS INCLUDED (EQN. A2.14). PARAMETER VALUES ARE GIVEN IN TABLE A2.4.

It is quite obvious that the red line in Fig. A2.23 significantly overestimates the observed values $\varphi(x)$ except for host pupae between 4 and 5 days old (corresponding to Q = 3.11 degree-days). To account for the difference between the expected and the observed values of $\varphi(x)$, we introduced a preference function $\pi(x)$ for ovipositing in pupae of age x allowing us to estimate $\varphi(x)$ adjusted for preference. The model reads

$$\varphi'(x) = \Phi(x)\pi'(x) \tag{A2.14}$$

where $\pi'(x)$ is the relative preference for pupae of age x given as $\pi'(x) = \frac{\pi(x)N(x)}{\sum_{x=0}^{\infty}\pi(x)N(x)}$.

$\Phi(x)$ is found as $\Phi(x) = \frac{\max\left(\varphi(x)\middle|N(x)>0\right)}{\max\left(\pi'(x)\right)}$, where $\max(\varphi(x) \mid N(x) > 0)$ is the maximum value of φ for the age groups represented in the population, i.e. all age groups for which $N(x) > 0$. The predictions based on Eqn. A2.14 are shown in Fig. A2.23 (black line) if all age groups are equally represented in the population.

$\pi(x)$ is modeled by means of the SANDY model as

$$\pi(x) = \pi_{\max}\left(\frac{Q(x)}{Q_{\max}}\right)^{a}\left(1-\frac{Q(x)}{Q_{\max}}\right)^{b} \qquad \text{(A2.15)}$$

where $Q(x)$ is the biological age of the host pupae. $\pi_{\max}$ by definition is 1, while the values of a and b are found in Table A2.4.

If all pupae are of the same age (x), $\varphi'(x)$ should be identical to $\varphi(x)$ (the red line in Fig. A2.23). This condition is fulfilled because $\pi'(x) = 1$ for that age group and 0 for all other age groups so that $\max\left(\varphi(x)\middle|N(x)>0\right) = \varphi(x)$ and $\max\left(\pi'(x)\right) = 1$. Note that Eqn A2.14 is appropriate to describe existing data, but needs to be tested experimentally with a more comprehensive data set consisting of different combinations of $N(x)$.

A2.3. Pesticide application

Figure A2.24 shows the fit of Eqn. A1.29 to data for Azadirachtin applied to larvae of stable flies (Miller and Chamberlain 1989). We have no data allowing us to estimate the decay rate of Azadirachtin in manure, so we assumed that $\delta = 0.1$ per day, corresponding to a half-time of 6.93 days (i.e. the time until the concentration in the manure has declined to 50% of the initial value).

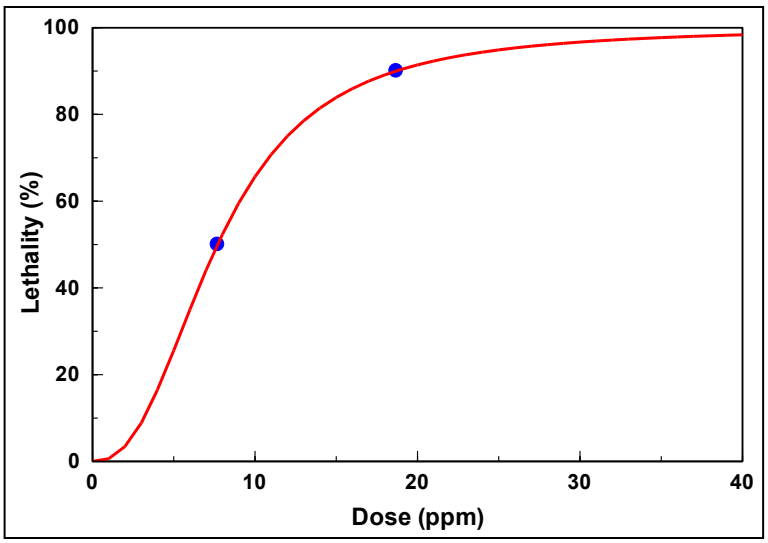

FIG. A2.24. DOSE-RESPONSE CURVE FOR AZADIRACHTIN APPLIED AGAINST STABLE FLIES. LINE IS THE FIT OF EQN. A1.30 TO DATA FROM MILLER AND CHAMBERLAIN (1989). THE PARAMETERS DESCRIBING LETHALITY ARE SHOWN IN TABLE A2.6.

A2.4. Environmental factors

A2.4.1. Temperature measurements

Temperature data measured outside and inside a stable are available from three farms located on Zealand (Table A2.1).

Farm	Coordinates	Period
Skibby	55°45'23.3"N, 11°59'20.1"E	1/4/1999 – 31/12/1999
Skibby	55°45'23.3"N, 11°59'20.1"E	1/4/2000 – 21/12/2001
Marke	55°41'52.2"N,11°27'4.3"E	1/3/2003 - 20/12/2004
Osted	55°32'56.4"N,11°56'54.0"E	10/3/2008 - 17/12/2008

TABLE A2.1. TEMPERATURE DATA AVAILABLE FROM THREE FARMS ON ZEALAND.

Because air temperature inside a stable was not measured during the winter time and we wanted to simulate the dynamics of the stable system for an entire year, we used a calibration model to estimate the missing temperatures from data obtained from nearby weather stations driven by the Danish Meteorological Institute (DMI). We first regressed the daily average air temperatures in the stable (T_{air}) against the corresponding values obtained from the nearest weather station (T_w) to find the calibration function given as

$$T_{air} = g(T_w) \tag{A2.16}$$

Figures A2.25 and A2.26 show the calibration lines for the farms in Marke and Osted. The relationships between T_w and T_{air} are adequately described by means of straight lines. The calibration models were used to estimate T_{air} from T_w during the periods where T_{air} was not measured directly.

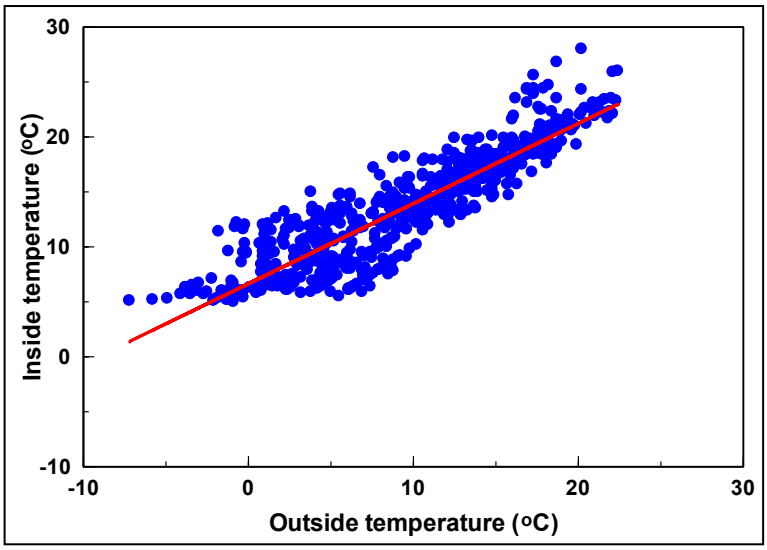

FIG. A2.25. THE RELATIONSHIP BETWEEN OUTSIDE AND INSIDE AIR TEMPERATURES IN MARKE. THE INSIDE TEMPERATURE (T_{AIR}) CAN BE ESTIMATED FROM THE OUTSIDE TEMPERATURE (T_W), MEASURED BY A WEATHER STATION, USING THE EQUATION $T_{AIR} = 6.6484 + 0.729T_W$ ($R^2 = 0.80$).

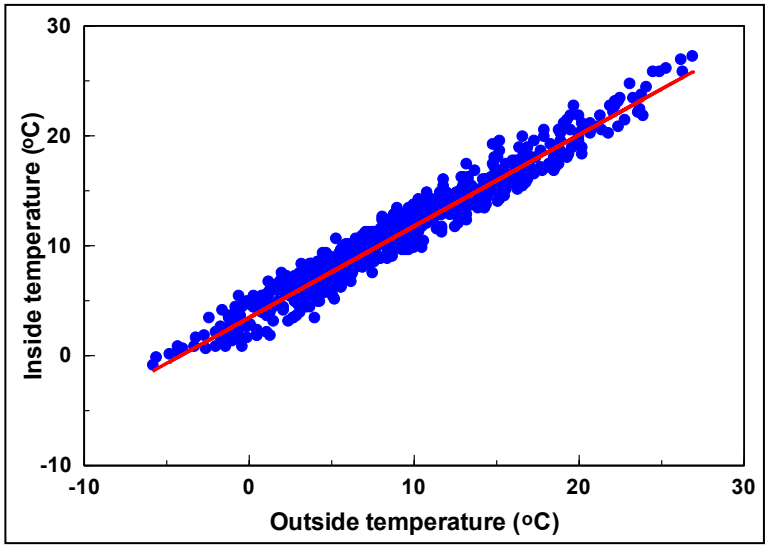

FIG. A2.26. THE RELATIONSHIP BETWEEN OUTSIDE AND INSIDE AIR TEMPERATURES IN OSTED. THE INSIDE TEMPERATURE (TAIR) CAN BE ESTIMATED FROM THE OUTSIDE TEMPERATURE (TW), MEASURED BY A WEATHER STATION, USING THE EQUATION TAIR= 3.4491 + 0.8323TW (R^2 = 0.967).

A2.4.2. Effect of depth on temperature in the manure

At three occasions (September 6, 2013, December 5, 2013 and March 14, 2014), we visited the stable in Marke to conduct temperature measurements. Manure temperatures were measured in an enclosure (ca. 3m x 3m) inhabited by 5-10 calves. 21 points were selected in a regular pattern to represent both the periphery and the center of the enclosure and at each point, temperature was measured at three depths (surface, 10 cm and 20 cm) by means of a thermistor (Tiny Tag loggers from Gemini). At the visit in September, manure depth was 20-35 cm, so measurements at all points could be made. In December manure depth was less than 20 cm at three of the points, so measurements could only be made at the surface and at 10 cm. Finally, at the third visit in March the stable had been cleaned recently, so no measurements were made.

The manure temperatures ranged between 20.1°C and 33.8°C on Sept. 6 and between 12.0°C and 35.8°C on Dec. 5. The average temperatures in the three depths at the two sampling occasions are given in Table A2.2.

Depth	September 6, 2013	December 5, 2013
Surface	26.96 (3.31) °C	23.81 (7.44) °C
10 cm	25.89 (3.22) °C	24.39 (7.94) °C
20 cm	24.33 (2.50) °C	23.13 (4.94) °C

TABLE A2.2. AVERAGES AND STANDARD DEVIATIONS (IN PARENTHESES) OF MANURE TEMPERATURES MEASURED IN THREE DEPHTS AT TWO OCCASIONS IN A STABLE IN MARKE. EACH VALUE IS BASED ON 21 OBSERVATIONS EXEPT AT 20 CM ON DECEMBER 5, WHERE *N* WAS 18

We used these six temperature measurements to calibrate the parameters of Eqn. A1.24, using the air temperatures in Marke from January 1, 2003 to December 31, 2004, to generate the manure temperatures in the same period. The air temperature on January 1, 2003, was 2.13°C. The floor temperature was assumed to be constant throughout the year and set to the average yearly temperature at Marke (12.97°C). The depth of the manure was assumed to be 30 cm and not to vary with time. The initial temperatures in all depths were set to the air temperature (2.13°C), while the parameters *c* and *K* were given some arbitrary initial values.

The model predicted manure temperatures after two years were then used as input for to initialize the manure temperatures in the next run instead of the air temperature and a new simulation performed. This was repeated until the initial and final temperature profiles were identical (typically after two to three runs). We then compared the agreement

between the predicted manure temperatures on Sept. 6 and Dec. 5 in both years with those measured in the surface (= 2 cm), 10 cm and 20 cm. The sum of squared deviations (SSD) between the predicted and observed temperatures was calculated. This procedure was repeated with a new combination of c and K to identify the combination with the lowest SSD (Table A2.6). The predicted manure temperatures in Marke are shown in Fig. 4.1.

A2.5. Estimation of larval competition parameter ψ

The only model parameter for stable flies that has not been estimated from independent experiments is ψ, which expresses the effect of larval density on larval survival rate (Eqn. A1.15). We therefore used the simulation model to estimate ψ as the value that yielded the best agreement between observed and predicted numbers of adult stable flies, because we did not have data on the abundance of larvae. The observed numbers of adult stable flies were estimated at sampling 22 occasions with approximately one month intervals in Marke from March 1, 2003, through December 31, 2004 (Skovgård & Nachman 2012). The predicted values of stable flies at the sampling dates were obtained from running the simulation model from January 1, 2003, through December 21, 2004. We have no recordings of the amount of manure and how often it was removed, so instead we assumed that the average depth of manure during the two years was 20 cm. As the manure depth affects the temperatures in the manure, the initial temperature profile corresponding to 20 cm manure was found by the procedure described in the previous section.

The predicted dynamics of stable flies were simulated using the manure temperatures obtained by the submodel described in Section A2.4 starting with an arbitrary value of ψ and an arbitrary number of stable flies in the four developmental stages. The numbers of individuals at the end of a simulation are relatively robust against the initial numbers. We therefore used the final numbers to initialize the following run, and repeated this procedure until the initial and final numbers were the same. The agreement between observed and predicted adult flies for a given value of ψ was assessed, and the procedure repeated for a new ψ (in steps of 1) to identify the value with the best fit. This was achieved with ψ = 20 cm^3/larvae (Fig. 4.1). The model explained 64% of the variation in the observed fly counts.

We plotted the observed values (y) against the predicted ones (x) and fitted the points with a straight line given as $y = a + bx$ (Fig. A2.27, broken line).Ideally, a and b should be 0 and 1, respectively. The fitted line has parameters a = 621 (SE = 8520) and b = 0.862 (SE = 0.134). a is not significantly different from 0 (t_{20} = 0.073; P = 0.943) and b is not significantly different from 1 (t_{20} = 1.032; P = 0.315) indicating that the simulation model satisfies the criterion that $y = x$ (Fig. A2.27, full line). The variation around the line is considerable, indicating that the model sometimes yields imprecise predictions, though it should be noticed that the observed values themselves are associated with considerable uncertainty. The largest deviation between observed and predicted values occurred on June 10, 2004, where the observed number of flies was 950 while the model predicts that the number should be 81950 (see Fig. 4.1). However, a few days earlier the predicted population size was much closer to the observed one. The largest discrepancies between observed and model predicted values occur when the population size changes steeply over a short period of time.

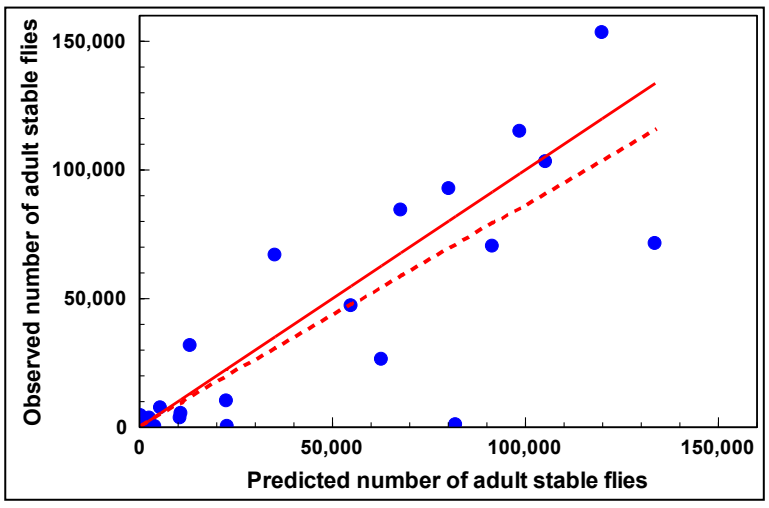

FIG. A2.27. OBSERVED NUMBER OF ADULT STABLE FLIES (Y)PLOTTED AGAINST THE MODEL PREDICTED VALUES (X). THE BROKEN LINE SHOWS THE FIT OF A STRAIGHT LINE TO DATA ($Y = 0.862X + 621$; $R^2 = 0.67$; $N = 22$) WHILE THE FULL LINE SHOWS $Y = X$ ($R^2 = 0.64$).

A2.6. List of parameter values used in the model

Description	Function	Stage	y_{max}	T_{min} /Q_{min}	T_{max} /Q_{max}	a	b
Effect of temperature on developmental rate	$\mathbf{S}(T)$	Eggs	[1]	11.26	43.43	1.2807	0.0240
			-	(0.014)	(-0.065)	(0.027)	(-0.002)
		Larvae	[1]	3.11	36.92	2.8422	0.3846
			-	(-0.078)	(-2.750)	(-1.016)	(0.704)
		Pupae	[1]	0.092	34.35	2.9185	0.2593
			-	(-0.001)	(-0.886)	(-0.586)	(0.478)
		Adults	[1]	9.60	45.00	8.4581	7.2629
			-	(-0.001)	(0.016)	(0.398)	(-0.357)
Effect of temperature on survival rate	$\mathbf{S}(s,T)$	Eggs	0.9847	6.31	{43.43}	0	0.0684
			(-0.970)	(<0.001)	-	(-0.037)	(0.069)
		Larvae	0.9886	{3.11}	{36.92}	0.0228	0.0656
			(4.977)	-	-	(0.147)	(-0.487)
		Pupae	0.9853	{0.092}	{34.35}	0.2409	0.1349
			(4.409)	-	-	(0.262)	(-0.387)
		Adults	0.9845	{9.60}	{45.00}	0.2161	0.4199
			(2.010)	-	-	(-0.195)	(0.407)
Effect of age on hatch rate	$\mathbf{S}(h,Q)$	Eggs	1	[0]	0.708	2.4559	[0]
			(-0.035)	-	(0.030)	(0.014)	-
		Larvae	0.7022	[0]	5.78	9.222	[0]
			(0.542)	-	(-2.181)	(-0.349)	-
		Pupae	0.9895	[0]	4.55	10.377	[0]
			(-0.026)	-	(-1.333)	(0.104)	-
Effect on age on mortality rate	$\mathbf{S}(d,Q)$,	Adults	[1]	[0]	27.02	1.7175	[0]
			-	-	(0.373)	(0.195)	-
Effect of temperature on fecundity rate	$\mathbf{S}(F,T)$	Adults	11.86	{9.60}	{45.00}	0.1579	0
			(-0.429)	-	-	(-0.187)	(0.052)
Effect of age on fecundity rate	$\mathbf{S}(F,Q)$	Adults	[1]	[0]	{27.02}	2.8967	6.1386
			-	-	-	(0.353)	(-0.188)
Sex ratio of offspring	$\mathbf{S}(f,T)$	Adults	0.5	{9.60}	{45.00}	[0]	[0]
			(-0.429)	-	-	-	-

TABLE A2.3. PARAMETER VALUES USED TO MODEL SURVIVAL, HATCHING AND MORTALITY OF *STOMOXYS CALCITRANS* BY MEANS OF THE SANDY MODEL. y_{max} CORRESPONDS TO THE MAXIMUM VALUE OF s, h, d AND F (I.E. s_{max}, h_{max}, d_{max} AND F_{max}). VALUES IN { } HAVE NOT BEEN ESTIMATED INDEPENDENTLY. VALUES IN [ ] ARE STRUCTURAL PARAMETERS, I.E. THE UNDERLYING FUNCTION IS ASSUMED TO BE EITHER A CONSTANT OR TO INCREASE MONOTONICALLY FROM 0 TO 1. BOUNDS HAVE BEEN IMPOSED ON THE TEMPERATURE PARAMETERS SO THAT $T_{min} \geq 0^{o}C$ AND $T_{max} \leq 45^{o}C$. VALUES IN ( ) SHOW ELASTICITIES.

Description	Function	Stage	y_{max}	T_{min} /Q_{min}	T_{max} /Q_{max}	a	b
Effect of temperature on developmental rate	**S**(T)	Immatures Adults	[1] - [1] -	10.08 (0.016) 0 (0.002)	35.00 (0.074) 45.00 (1.804)	1.5394 (-0.088) 2.2414 (0.759)	0.1053 (0.028) 0.00024 (-0.002)
Effect of temperature on survival rate	**S**(s,T)	Immatures Adults	0.9943 (-8.565) 1 (2.768)	{10.07} - {0} -	{35.00} - {45.00} -	0.0020 (0.055) 0.0593 (0.003)	0.0078 (-0.169) 0.0413 (-0.003)
Effect of age on hatch rate	**S**(h,Q)	Immatures	1 (0.019)	[0] -	21.06 (-0.119)	7.2560 (-0.043)	[0] -
Effect on age on mortality rate	**S**(d,Q),	Adults	[1] -	[0] -	12.26 (0.670)	3.2712 (0.180)	[0] -
Effect of temperature on attack rate*)	**S**(E,T)	Adults	18.3595 -	{0} -	{35.00} -	2.8465 -	0.0624 -
Effect of parasitoid age on attack rate	**S**(E,Q)	Adults	[1] -	{0} -	{12.26} -	0.5454 (0.199)	4.2991 (-0.488)
Effect of temperature on success ratio*)	**S**(φ,T)	Adults	0.6228 -	0 -	35.00 -	0.0092 -	0.0422 -
Effect of parasitoid age on success ratio	**S**(φ,Q)	Adult	[1] -	{0} -	{12.26} -	0.0035 (0.024)	0.7003 (-0.096)
Effect of temperature on attack rate¶)	**S**(E,T)	Adults	20.230 (0.686)	8.07 (-0.171)	36.56 (-0.593)	4.3423 (-0.361)	1.6921 (0.313)
Effect of temperature on success ratio¶)	**S**(φ,T)	Adults	0.8998 (0.676)	15.00 (-0.021)	35.47 (-0.012)	0.2938 (-0.014)	0.1737 (0.011)
Effect of host age on attack rate†)	**S**(v,Q)	Adults	[1] -	{0} -	{4.55} -	0.4343 (0.476)	0.1443 (-0.137)
Effect of host age on host preference†)	**S**(π,Q)	Adults	[1] -	{0} -	{4.55} -	13.062 (-3.834)	5.6982 (0.511)
Sex ratio of offspring	**S**(f,T)	Adults	0.707 (0.687)	{15.00} -	{25.47} -	[0] -	[0] -

TABLE A2.4. PARAMETER VALUES USED TO MODEL SURVIVAL, HATCHING AND MORTALITY OF *SPALANGIA CAMERONI* AND ITS INTERACTIONS WITH PUPAE OF S. CALCITRANS BY MEANS OF THE SANDY MODEL. SEE TABLE A2.3 FOR FURTHER EXPLANATION.
*) VALUES ARE BASED ON LIFE-TABLE DATA USING HOUSE FLY PUPAE AS HOSTS (SKOVGÅRD & NACHMAN SUBM.). THESE VALUES ARE NOT USED IN THE SIMULATION MODEL.
¶) VALUES ARE BASED ON FUNCTIONAL RESPONSE DATA USING STABLE FLY PUPAE AS HOSTS (SKOVGÅRD & NACHMAN 2015A). THESE VALUES ARE USED IN THE SIMULATION MODEL.
†) Q IS THE BIOLOGICAL AGE OF THE HOST (LARSEN 2006).

Description	Symbol	Value	Dimension
Effect of host density on survival rate	ψ	20 (-1.026)	cm^3/host larvae
Searching efficiency	α	1.1196 (0.734)	cm^2/host pupae
Functional response type	β	1 (-3.476)	Dimensionless
Effect of parasitoid density on attack rate	r	2.6202 (-0.070)	cm^2/parasitoid female
Effect of parasitoid density on attack rate	q	1.6663 (0.381)	Dimensionless
Effect of parasitoid density on success ratio	r	0.2030 (-0.164)	cm^2/parasitoid female
Effect of parasitoid density on success ratio	q	0.0279 (0.003)	Dimensionless

TABLE A2.5. PARAMETER VALUES USED TO MODEL INTRASPECIFIC COMPETITION AMONG *S. STOMOXYS* LARVAE AND ADULT *S. CAMERONI* FEMALES. VALUES BASED ON SKOVGÅRD & NACHMAN (2015AB). VALUES IN PARENTHESES GIVE ELASTICITIES.

Description	Symbol	Value	Dimension
Lethality of Azadirachtin	μ_0	-5.0547	Dimensionless
Lethality of Azadirachtin	μ_1	5.7019	Dimensionless
Decay rate of Azadirachtin	δ	0.1	day^{-1}
Average amount of manure left after cleaning	m_0	1	cm
Heat production rate due to fermentation	K	0.09 (0.804)	^oC/day
Heat conduction rate	c	0.47 (-0.789)	day^{-1}
Floor temperature	T_{floor}	12.97 (0.361)	^oC
Upper surface layer (0-L_1 cm)	L_1	3 (0.128)	cm
Lower surface layer (0-L_2 cm)	L_2	8 (1.751)	cm

TABLE A2.6. MISCELLANEOUS PARAMETERS. VALUES IN PARENTHESES GIVE ELASTICITIES (ONLY COMPUTED FOR THE PARAMETERS ASSOCIATED WITH THE BIOLOGICAL CONTROL STRATEGY)

Appendix 3: A user manual to *The Fly Simulator*

A user manual to

The Fly Simulator

A simulation model of biological control of stable flies by means of parasitoids.

By Gösta Nachman and Henrik Skovgård

List of content

A3.1. Introduction to *The Fly Simulator*

The Fly Simulator is an interactive computer program allowing you to simulate the interactions between stable flies (*Stomoxys calcitrans*) and its natural enemy, the parasitoid *Spalangia cameroni*. The simulation program is based on a mathematical description of the system presented in a scientific report entitled *Modelling biological control of stable flies by means parasitoids*. The report, as well as *The Fly Simulator* program, can be freely downloaded from http://mst.dk/.

The estimated parameters used in the model are thoroughly described in the above-mentioned report. They serve as the default parameter values in *The Fly Simulator* program, but the program allows you to run simulations with other parameter values.

The default scenario in *The Fly Simulator* mimics a Danish farm located in Marke, Zealand, Denmark. It is an organic dairy farm with approximately 60-70 milking cows and about 35-50 calves. The calves are housed in a separate building, where they move freely on straw bedding mixed with manure, which is an excellent substrate for development of stable flies. The abundance of adult stable flies has been surveyed by means of a mark-recapture method (Skovgård & Nachman 2012, Nachman & Skovgård 2012) once every month through a period of two years. The daily air temperature inside the stable was recorded through the same period.

The Fly Simulator program allows you to simulate the dynamics of stable flies at the farm based on the recorded daily temperatures and to compare the predicted abundance of flies with the observed abundance. The default scenario simulates the stable flies if no measures are taken control them. It will be up to you to apply different control methods (parasitoids, pesticides, cleaning) to find out how efficiently you can apply these methods, either singly or in combination, to control the flies. The program also enables you to compare the different control methods by converting them to a common currency (Danish Crowns (DKK)), which allows you to make a cost-benefit analysis, i.e. how to obtain the highest reduction in stable flies per invested DKK.

A3.2. What is required to run *The Fly Simulator?*

The Fly Simulator is one among several models embedded in the program called *Fitom* (**F**rom **I**ndividuals **to** **M**etapopulations). In this manual we only describe how to use *The Fly Simulator*. You are welcome to test the other models, but there is no guarantee that they will run smoothly on your computer as some of them are still under development.

The program requires a PC with Windows (XP, 7 and8, but newer versions of Windows might also work). After downloading it to your computer, double-click the setup file, which will automatically install *Fitom* on your computer's C-drive and the Fitom icon

will appear on your desktop. If error messages should turn up during installation, use "Ignore" to continue installation.

A3.3. How to use *The Fly Simulator*?

A3.3.1. Run your first simulation

(1) Double-click the Fitom icon and the front page will appear (Fig. A3.1)

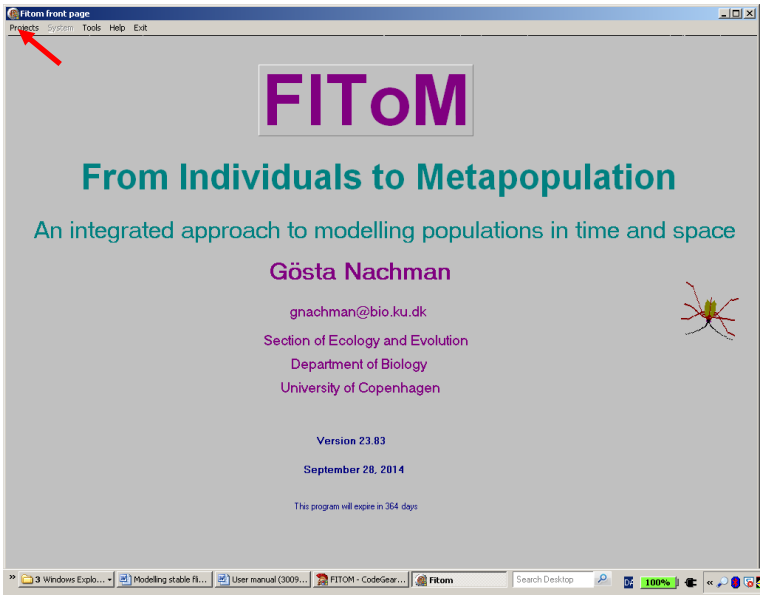

FIG. A3.1. THE FRONT PAGE OF FITOM.

(2) Click on **Project** and choose the project called **Fly Simulator.** The front page of *The Fly Simulator* model will now appear (Fig. A3.2).

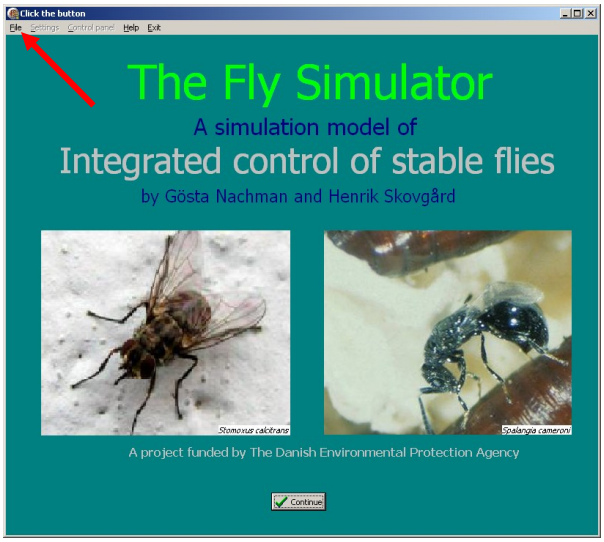

FIG. A3.2. THE FRONT PAGE OF THE *FLY SIMULATOR* MODEL.

(3) Click on either **Continue** or **File** to open the input file dialog (Fig A3.3). Choose the folder called "Data" and open the default scenario in the file called "FlySimulator.fit" located in the folder "C:\Program files\Fitom MST\FlySimulator\Data"

.

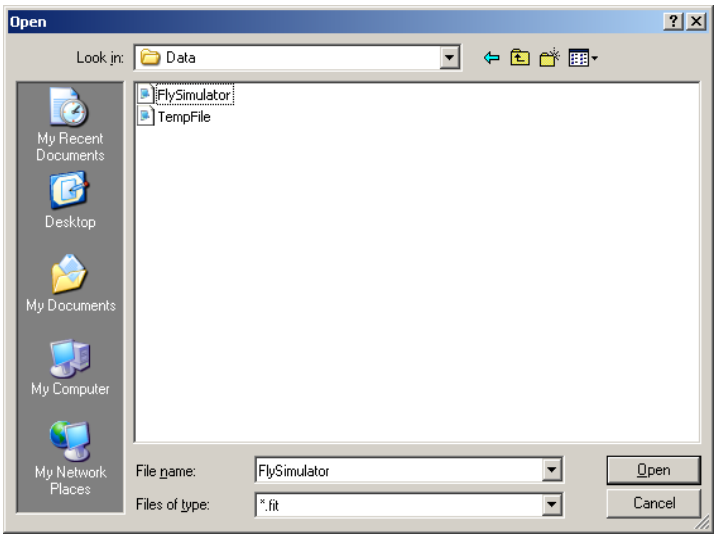

FIG. A3.3. THE DIALOG BOX TO OPEN AN INPUT FILE.

(4) You now have access to two new forms, called the *Fly Simulation Model* showing the flow chart of the model and the *Control Panel* (Fig. A3.4). The *Fly Simulation Model* allows you to change the initial population sizes and to the settings for the model, while the *Control Panel* is used to interact with the system during a simulation.

(5) If you move or close the *Fly Simulation Model* you will see the underlying form called *System*, which is the visual interface to the model during a simulation. The boxes in the left side of the form show information about the current state of the system. Use the mouse to point at a box if you want to know more about its content.

(6) Click on the **Run** button on the *Control Panel* to start a simulation with the default values. To stop a simulation, press **Stop**.

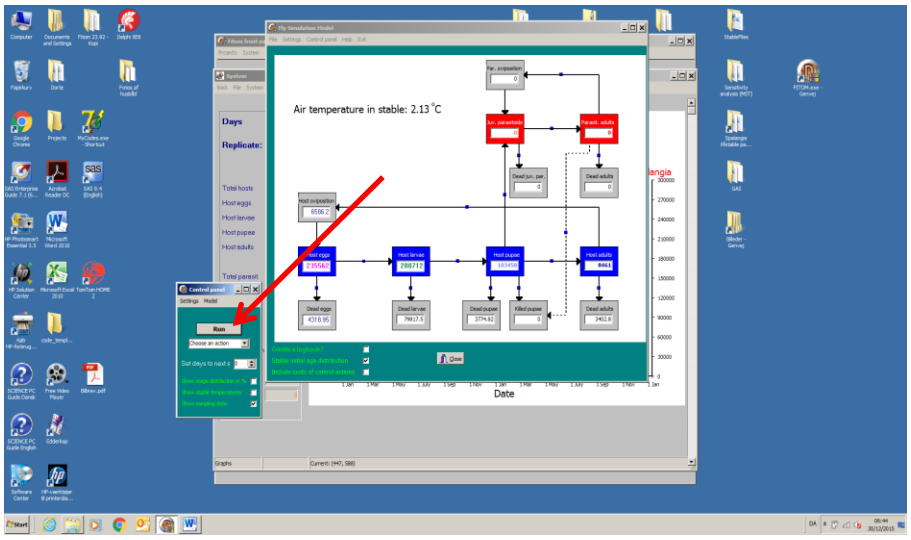

FIG. A3.4. THE FLOW CHART OF *THE FLY SIMULATOR* MODEL AND THE *CONTROL PANEL*.

(7) When you have finished a simulation, you will need to clear the graphs by pressing the **Clear graphs** button on the *Control Panel* (Fig. A3.5). You will be asked whether you are sure that the graphs have to be cleared. If you want to return to the *Fly simulation model,* you can open the form by clicking on the **Model** button on the *System* form or on the *Control Panel*.

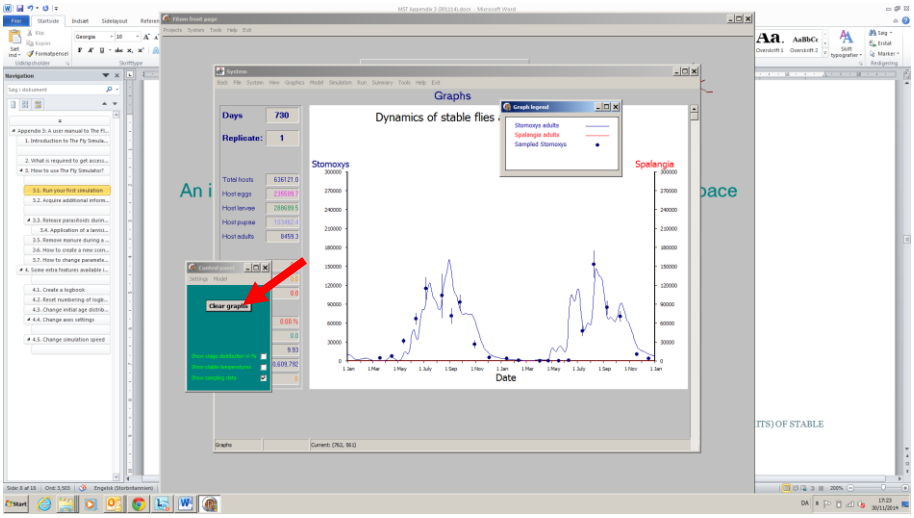

FIG. A3.5. THE OUTPUT OF THE DEFAULT SIMULATION. THE POINTS SHOW THE OBSERVED NUMBER (±95% CONFIDENCE LIMITS) OF STABLE FLIES ON A CATTLE FARM IN MARKE AND THE LINE THE PREDICTED POPULATION SIZE OF STABLE FLIES.

(10) You can leave the program again by clicking on the **Exit** button in the menu of the *System* form.

A3.3.2. Acquire additional information about the system during a simulation

(1) If you put a tag in the box called **Show stage distribution in %** on the *Control Panel* the populations in the info boxes on the *System* form will be shown in per cent instead of absolute numbers.

(2) If you put a tag in the box called **Show stable temperatures** on the *Control Panel* two new forms appear on the screen, showing information about the two stable compartments occupied by cattle. Apart from showing temperature in the air and at different depths in the manure, the *Compartment* forms also provide information about the amount of manure (in kg), the depth (in cm) of the manure and the concentration of pesticide (in ppm) on the *Info* sheet (Fig. A3.6).

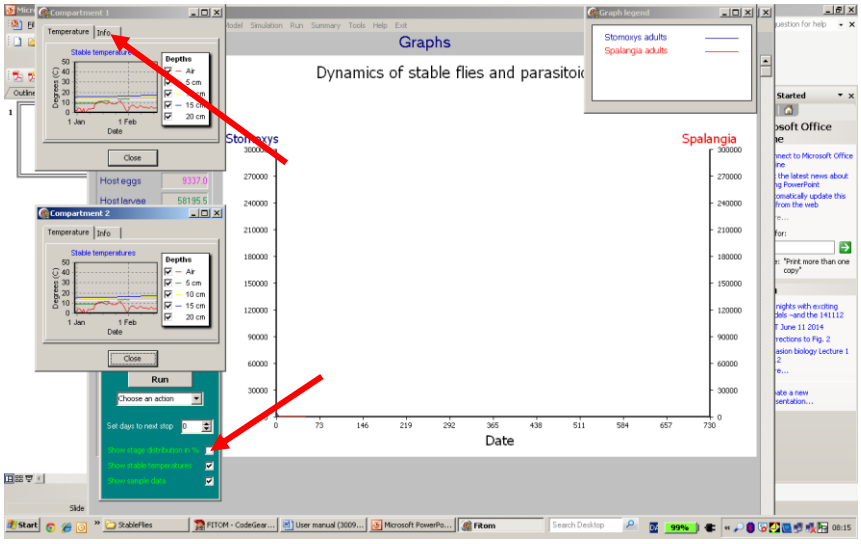

FIG. A3.6. THE *COMPARTMENT* FORMS APPEARING WHEN TAGGING THE BOX CALLED "SHOWING THE STABLE TEMPERATURES" ON THE *CONTROL PANEL*.

(3) If you remove the tag in the box called **Show sampling data**, the sampling data (if they exist) will no longer appear during a simulation.

(4) If you stop the model during a simulation and click on the **View** button in the menu (Fig. A3.7), you can choose between three different views. The default view is the **Graph** view.

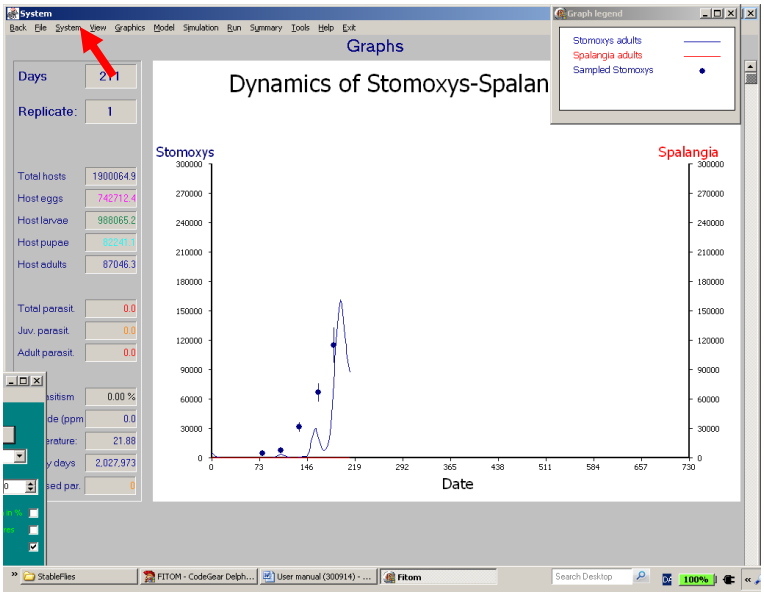

FIG. A3.7. THE VIEW BUTTON ENABLES YOU TO SWITCH BETWEEN VIEWS.

The **2D view** shows the number of individuals in each of the two compartments as dots in colors reflecting their species and stage (Be patient, it may take some time to generate a 2D view). The **3D view** shows the same information but as stacked bars with heights proportional to the abundance of each stage (see section A3.3.6 to change the colors used to visualize stages within species). Finally, if you use the 2D view and click with the mouse on one of the compartments, you can get information about the number of individuals in each stage of each species in the chosen compartment (called a patch in the form) (Fig. A3.8).

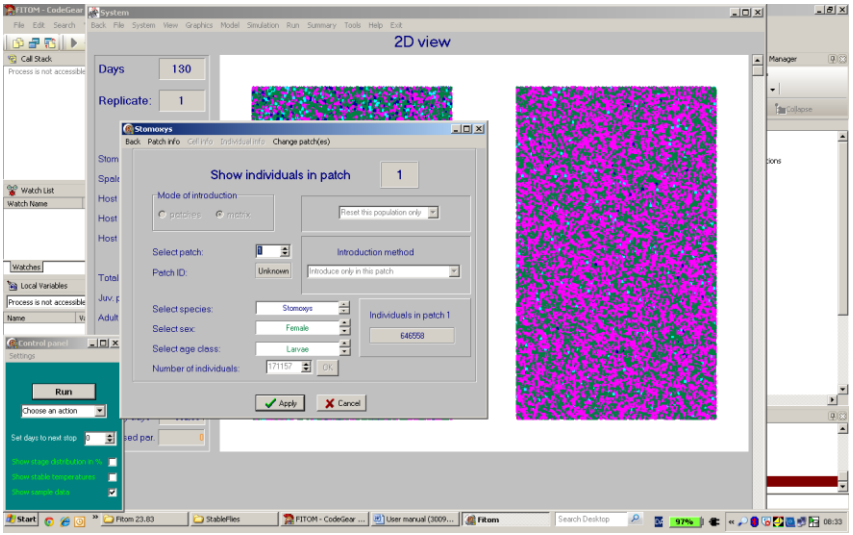

FIG. A3.8. THE 2D VIEW SHOWING COMPARTMENT 1 TO THE LEFT AND COMPARTMENT 2 TO THE RIGHT OVERLAID WITH AN INFO APPEARING WHEN YOU POINT AT A COMPARTMENT (CALLED A PATCH IN THE FORM)

A3.3.3. Release parasitoids during a simulation

(1) On the *Control Panel* set the number of days to e.g. 150 days and start the simulation. When the simulation stops, you can choose an action from the **Action options** list (Fig. A3.9).

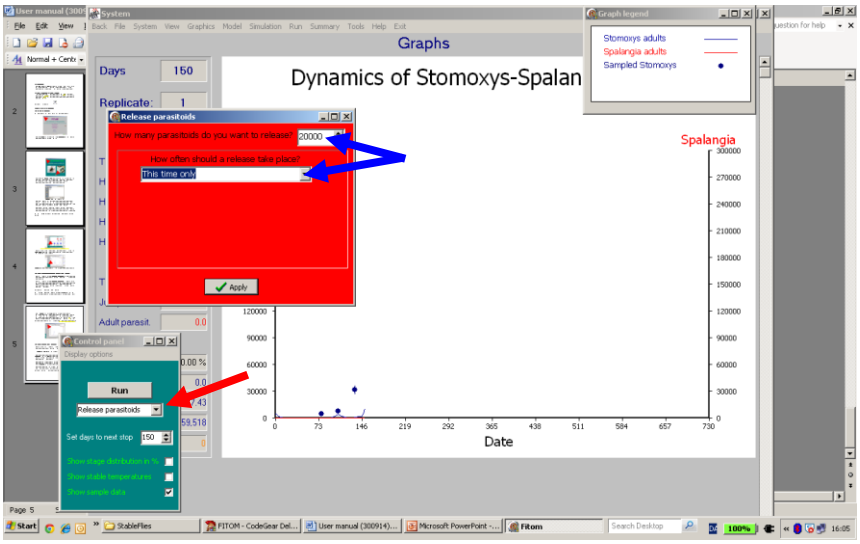

FIG. A3.9. THE ACTION LIST ALLOWS YOU TO INTERFERE WITH THE SYSTEM WHEN THE SIMULATION IS STOPPED.

(2) Choose "Release parasitoids" from the **Action options** list. A new form called *Release parasitoids* appears. Choose to release e.g. 20,000 parasitoids and "This time only" from the **Release options** list. Click on the **Apply** button and resume the simulation. You can repeat releases of parasitoids several times during a simulation. The total number of released parasitoids appears on the *System* form.

(3) Another release method is to choose the option "Release parasitoids several times until end of simulation" from the **Release options** list and then choose the interval between the automatic releases.

(4) A third option is to release parasitoids only during periods where stable flies use to be a nuisance (typically from May to October). Choose "Several times during a chosen period" and choose the starting and ending date of the period as well as the time between the releases. This option should be implemented before a simulation starts.

(5) A fourth option is to release parasitoids each time the number of stable flies exceeds a threshold. Choose "Apply an adaptive release strategy". You can then define the threshold value and the minimum interval between releases.

(6) When you start a new simulation, the release method applied in the previous simulation will remain unless you reset the action(s) by choosing "Reset all actions" in the **Action options** list on the *Control Panel*.

A3.3.4. Application of a larvicide treatment during a simulation

(1) If you have already used a control strategy, you can reset previous actions by choosing "Reset all actions" in the **Action options** list on the *Control Panel*. Then choose "Apply a larvicide" from the **Action options** list. The *Pesticide application* form now appears (Fig. A3.10).

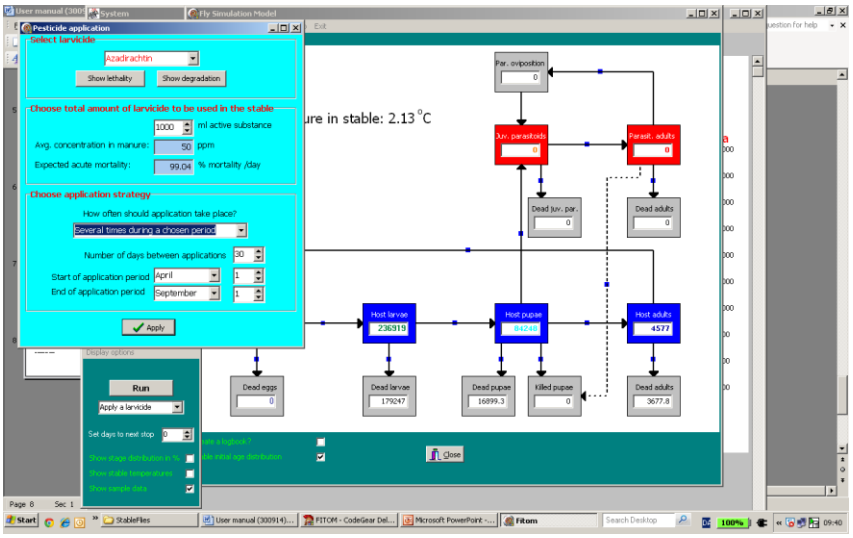

FIG. A3.10. THE *PESTICIDE APPLICATION* FORM OPENED BY CHOOSING THE PESTICIDE APPLICATION OPTION IN THE ACTION OPTIONS LIST ON *CONTROL PANEL*

(2) Choose a pesticide from the **Pesticide options** list (at the moment the only option is Azadirachtin). By clicking on the **Show lethality** and **Show degradation** button you get information about the chosen pesticide's dose-lethality and degradation curves, respectively. The current average concentration of pesticide in the manure is shown on the *System* form.

(3) Similar to the procedure used for releasing parasitoids, you can choose the release strategy from the **Application options** list.

A3.3.5. Remove manure during a simulation

(1) The default scenario assumes that the amount of manure in the stable is constant. In order to simulate how removal of manure will affect population dynamics, it is necessary to include that new manure is produced in the stable so that manure will accumulate over time until the stable is cleaned. Click on **Settings** in the menu of either the *Fly Simulation Model* form or the *Control Panel* and choose the button **Stable** in the sub-menu to get direct access to the *Stable* sheet where changes in the settings associated with the stable can be made (Fig. A3.11). Change "Manure production" in Compartment 1 to e.g. 60 kg/day and in Compartment 2 to e.g. 70 kg/day. The values reflect the number of animals and their size in the respective compartments.

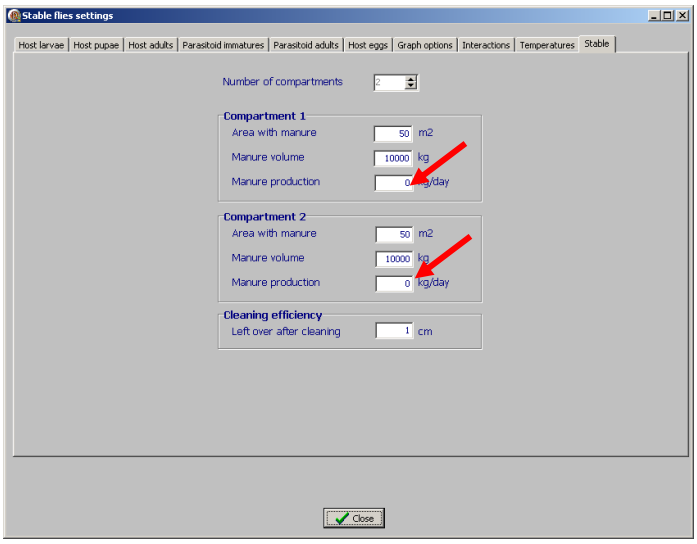

FIG. A3.11. THE *STABLE* SHEET ALLOWING YOU TO MODIFY THE STABLE.

(2) Now go the *Control Panel*. If you have already used a control strategy, you can reset the previous actions by choosing "Reset all actions" in the **Action options** list on the *Control Panel*. Then choose "Remove manure" from the **Action options** list. The *Manure removal* form now appears (Fig. A3.12).

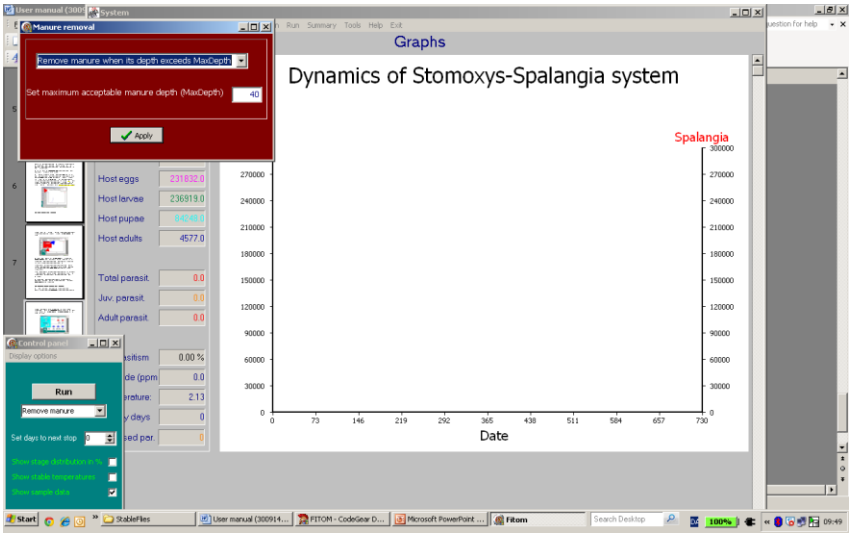

FIG. A3.12. THE *MANURE REMOVAL* FORM OPENED BY CHOOSING THE REMOVE MANURE OPTION IN THE ACTION OPTIONS LIST ON *CONTROL PANEL*

(3) Choose how removal of manure should take place in the **Manuring options** list on the *Manure removal* form. You can only remove the manure from one of the two compartments on a given day. The option "Remove manure when its depth exceeds MaxDepth" removes manure automatically from the compartment where the depth of manure exceeds the specified maximum depth. Removal of manure kills the eggs, larvae and pupae of both species, as well as the adult parasitoids in proportion to the amount of removed manure (i.e. 90% are killed if 90% of the manure is removed). Adult stable flies escape removal of manure.

A3.3.6. How to create a new scenario and save it

A scenario consists of the initial state of the system and the parameters of the mathematical equations describing how the system's state variables vary over time. In section A3.3.5 we changed the default scenario from one with a constant amount of manure to one where manure will accumulate over time. In this section we will make more extensive changes in the initial state of the system and save it as a new scenario.

(1) Start the program and open the default project (Section A3.3.1). Click on **Settings/Temperatures** and then on the **Temperature scenario options** box and choose "Osted March 11 to December 17, 2008". Run the simulation with temperature data from this stable. Then clear the graphs.

(2) Instead of just showing the number of adult stable flies when you run a simulation, you may decide to see the dynamics of all or some of the other stages. Click on **Settings/Graph options** and then set a tag outside the stages you want to be depicted. Try for instance to mark all stages of stable flies (Fig. A3.13). You can also change the default colors. Run the simulation. Numbers exceeding 1,000,000 are displayed on the Y-axes as x.xEy which corresponds to $x.x \cdot 10^y$. For instance, 1.2E6 corresponds to 1,200,000.

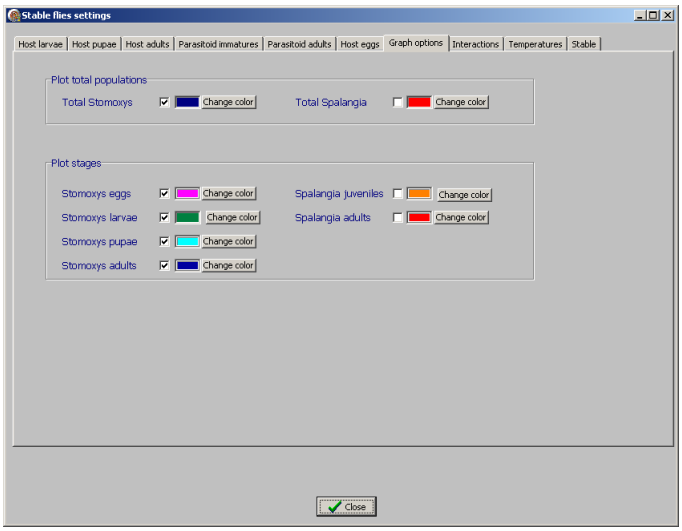

FIG. A3.13. THE GRAPH OPTIONS SHEET

(3) Figure A3.14 shows the final state of the system. As you can see, the predicted numbers of stable flies are ca. 440,492 eggs, 157,235 larvae, 95,246 pupae, and 4,891 adults. To see the initial numbers clear the graphs and open the model form (click on **Model** on the *System* form). Try to change the initial numbers of stable fly eggs, larvae, pupae and adults in the blue boxes. Note that the rates in the grey boxes are automatically updated in accordance with model's parameter values. Run the simulation with the new initial numbers.

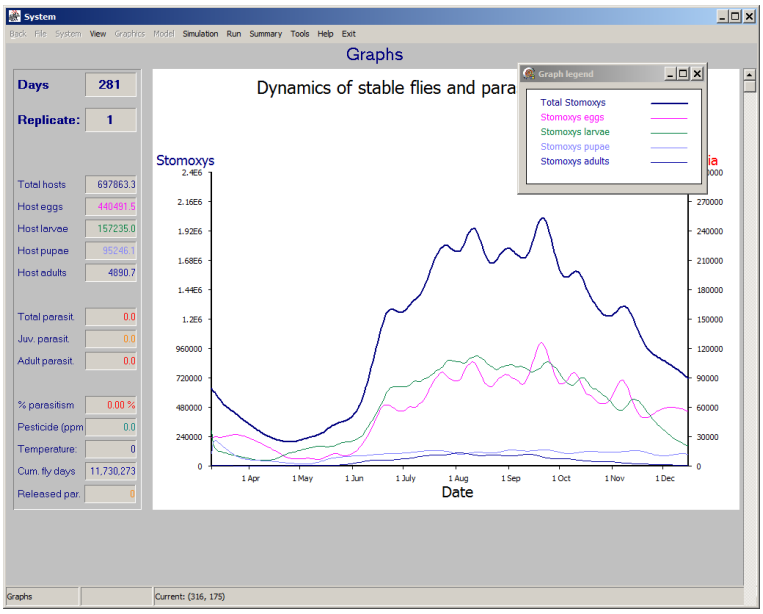

FIG. A3.14. PREDICTED DYNAMICS OF STABLE FLIES IN OSTED FROM MARCH 17 THROUGH DECEMBER 17, 2008.

(4) You can save the changes you have made to a scenario by clicking on the **File** button either on the *System* form or on the *Fly simulation model* form. If you choose **Save scenario,** you will be asked whether you are sure that you really want to overwrite the existing scenario with the new scenario. If you answer no, you should choose **Save scenario as** and the *Save as* box appears (Fig. A3.15). Give the file containing the scenario a new name, for instance "FlySimulator1" and then press the **Save** button. It will be saved in the directory called "...\FlySimulator\Data\" together with all the other files associated with the scenario (these files have the same name but different extensions).

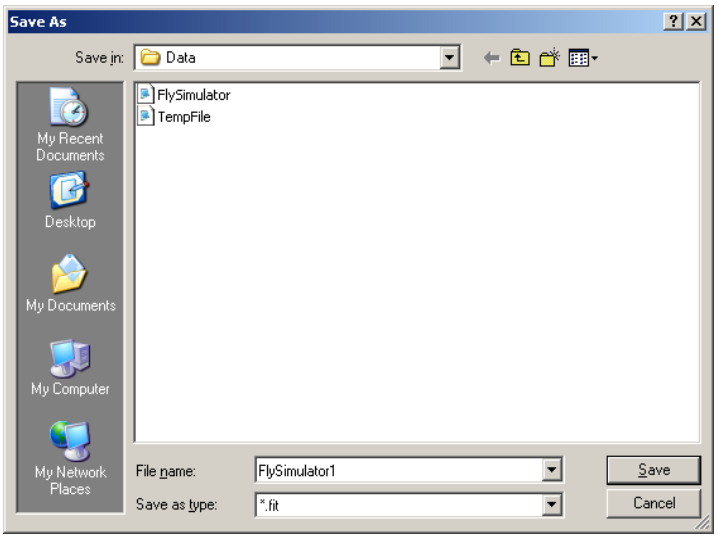

FIG. A3.15. THE *SAVE AS* DIALOG BOX TO BE USED WHEN YOU WANT TO SAVE A NEW SCENARIO IN A NEW FILE NAME (HERE CALLED "FLYSIMULATOR1". THE SCENARIO WILL AUTOMATICALLY BE ASSIGNED FILE NAME EXTENSIONS.

A3.3.7. How to change parameter values

(1) In case you want to use *The Fly Simulator* with other parameter values than the default, you can change parameter values. This can be done in two ways: (a) Click at a blue square on an arrow in the flow chart on the *Fly Simulation Model* form and you will get access to the parameters determining this relationship; (b) Click on the **Settings** button and then choose the relevant parameter(s).

You can see how a change in a parameter value affects the function in which the parameter occurs when you click on **Show function** button. In some cases, the function includes the combined effects of several factors. You can see how the graph looks like at another temperature or another parasitoid density by changing the values in the boxes at the bottom of the form (Fig. A3.16).

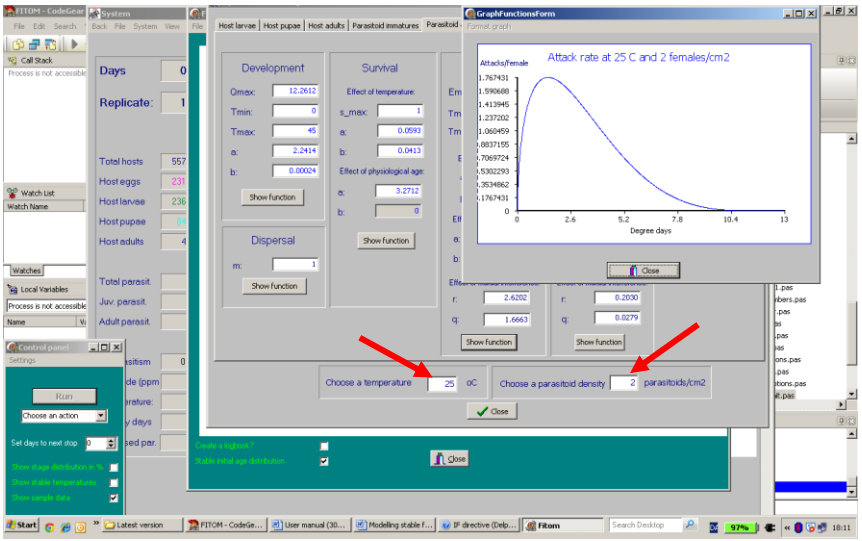

FIG. A3.16. EFFECT OF AGE (IN DEGREE-DAYS), TEMPERATURE, AND PARASITOID DENSITY ON THE ATTACK RATE OF AN INDIVIDUAL ADULT FEMALE PARASITOID. THE GRAPH DEPICTS THE ATTACK RATE AT 25^{0}C AND 2 ADULT PARASITOIDS PER CM^{2}.

A3.3.8. How to include the costs of controlling flies into the model analysis.

In order to optimize control of stable flies, it is necessary to know the costs associated with the various control options. Unfortunately, such information is still not available. Nevertheless, we have included an option in *The Fly Simulator* to set a price on the fixed and variable costs associated with releases of parasitoids, application of larvicide and removal of manure. The option offers some default values of these costs, but it has to be emphasized that they are more or less arbitrary, so it will be up to the user to change the values if he/she disagrees. In order to optimize control, it is necessary to run at least two runs: one in which no control is implemented and one in which some control measures are taken. The criterion for optimizing control is to achieve a certain control index (*CI*) (e.g. 80% reduction in *FlyDays*) at the lowest possible costs (in DKK) for each killed fly.

(1) On the model form set a tag at "Include costs of control actions" (Fig. A3.17).

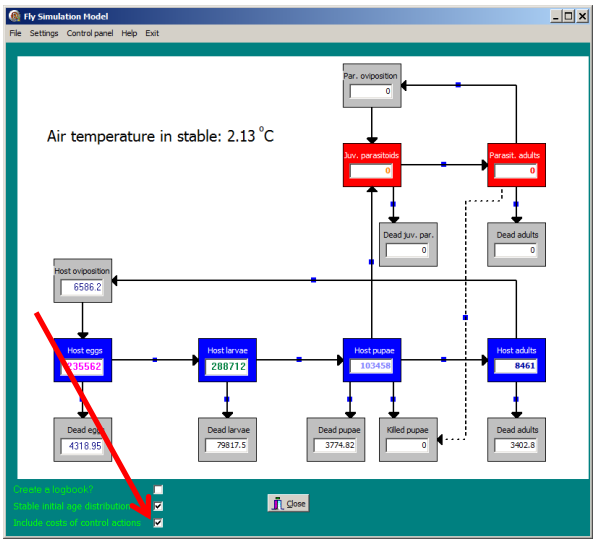

FIG. A3.17. THE OPTION FOR INCLUDING COSTS OF CONTROLLING STABLE FLIES

(2) If you now click on **Settings/Costs**, you get access to the parameters associated with the costs of applying biological, chemical and physical control (Fig. A3.18). Fixed costs are expenditures associated with, e.g., ordering, paying, transporting, storing and applying parasitoids and insecticides or, in the case of manuring, investment in and maintainance of machinery. The variable costs refer to expenses that increase with the efforts invested in control, i.e., number of released parasitoids, dose of insecticide, and amount of manure removed. Note that the default values shown in Fig. A3.18 are arbitrary and just used for illustrative purposes.

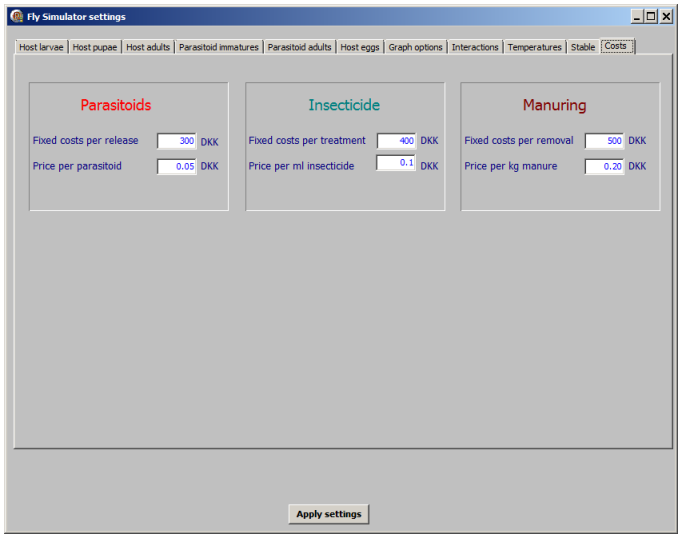

FIG. A3.18. THE DEFAULT VALUES OF COSTS ASSOCIATED WITH VARIOUS CONTROL OPTIONS

(3) Now choose the settings for **Stable** and apply the settings shown in Fig. A3.19. Thus, it is assumed that the calves in compartment 1 and 2 produce 50 and 60 kg kg manure per day, respectively.

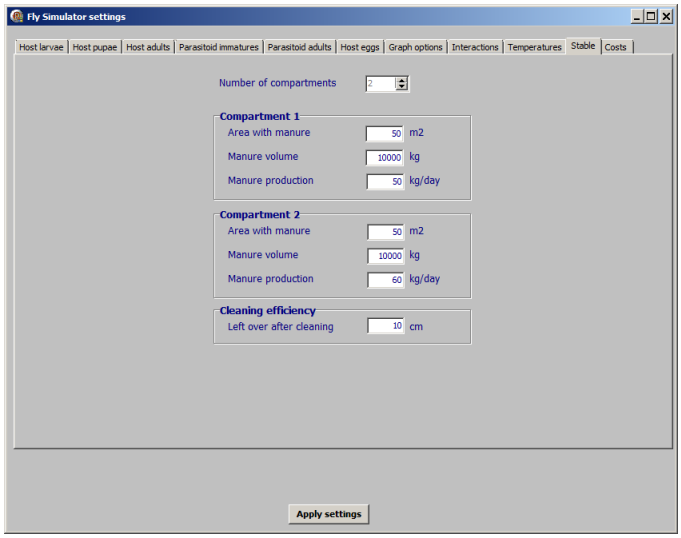

FIG. A3.19. PARAMETERS CHARACTERIZING THE TWO COMPARTMENTS IN THE STABLE.

(4) Now click on **Start** and run a simulation without any interference. At the end of the simulation, a form called *Evaluation of stable fly control* appears (Fig. A3.20). It shows that the number of *FlyDays* in the example exceeds 45,000,000 (corresponding to approximately 62,000 flies per day on average).

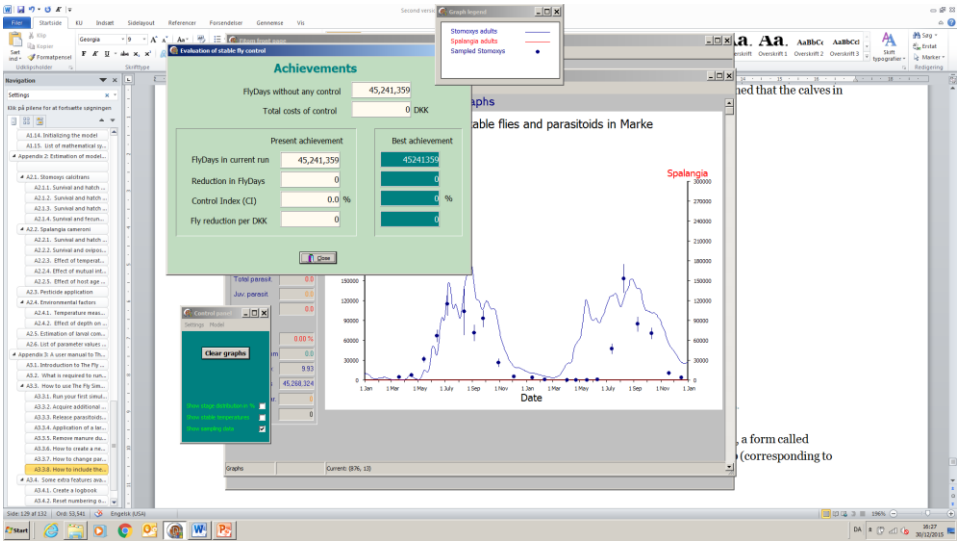

FIG. A3.20. MAXIMUM NUMBER OF FLYDAYS IF NO MEASURES ARE TAKEN AGAINST REDUCING ABUNDANCE OF STABLE FLIES.

(5) Clear the graphs and plan a new simulation where you remove manure each time the depth exceeds 35 cm. Fig. A3.21 shows that the estimated costs associated with this action appears on the *Manure removal* form.

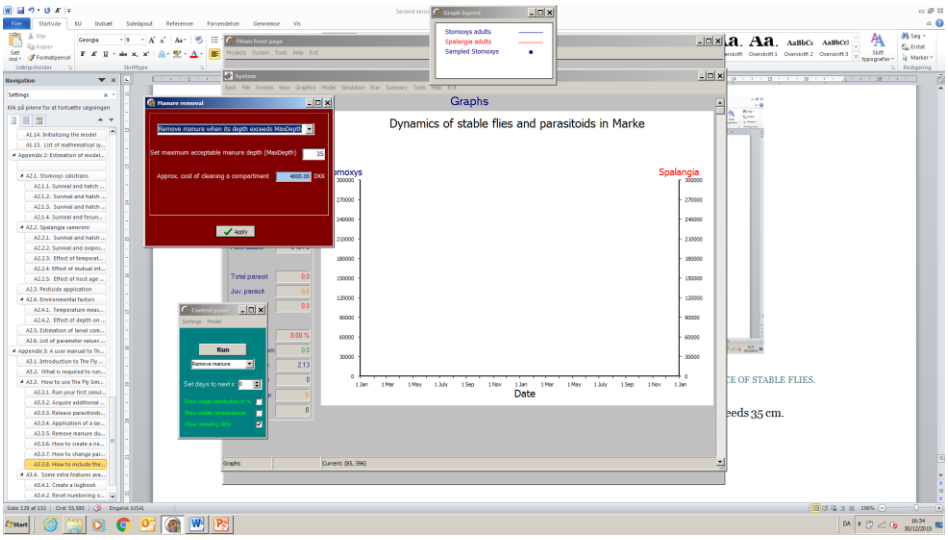

FIG. A3.21. MANURE IS REMOVED EACH TIME THE DEPTH IN A COMPARTMENT EXCEEDS 35 CM. THE ESTIMATED COST IS DKK 4,000 EACH TIME REMOVAL IN A COMPARTMENT TAKES PLACE.

(6) Figure A3.22 shows the outcome of the proposed simulation. The total costs of manuring during the two years are seen to be DKK 18,124. The number of *FlyDays* is 19,208,419 which represents a decline of 57.5% compared with an untreated stable. The reduction corresponds to 26,032,940 *FlyDays*. For each DKK invested in control, *FlyDays* decreased with 1436.4 flies. As this was the first attempt to control stable flies, the results also represent the best achievement so far.

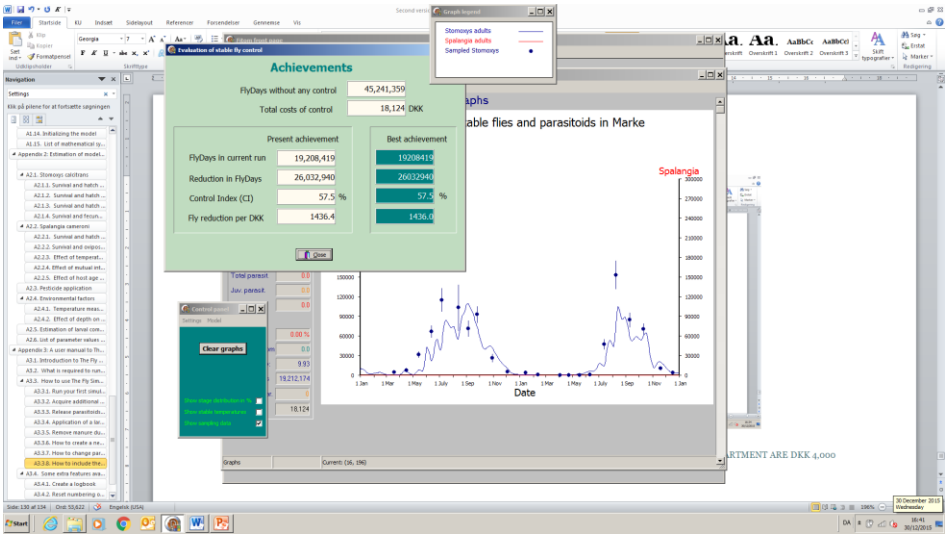

FIG. A3.22. RESULTS OF REMOVING MANURE EACH TIME ITS DEPTH EXCEEDS 35 CM

(7) You can now try to improve the results shown in Fig. A3.22 by combining manuring with biological and/or chemical control (as long as you do not “Reset all actions”, the strategy from the previous run will also be performed in the new run). The *Release parasitoids* and the *Pesticide application* forms show the estimated costs associated with the chosen control strategy. We suggest that you try to find the best strategy that keeps the control index (CI) above 80% at the lowest possible cost (i.e. to maximize fly reduction per DKK). You may apply a strategy based on both chemical and biological control (iutegrated control) or try to avoid using insecticide. The column to the right on the evaluation form gives the best result achieved so far.

A3.4. Some extra features available in *The Fly Simulator*

A3.4.1. Create a logbook

When you have opened a scenario (Section A3.3.1) you may want to store information about individual simulations without having to save every scenario separately. The logbook can be used to store your personal notes and other information about the individual simulations. To create a logbook, put a tag in the box called **Create logbook?** on *The Fly Simulator model* form (Fig. A3.23).

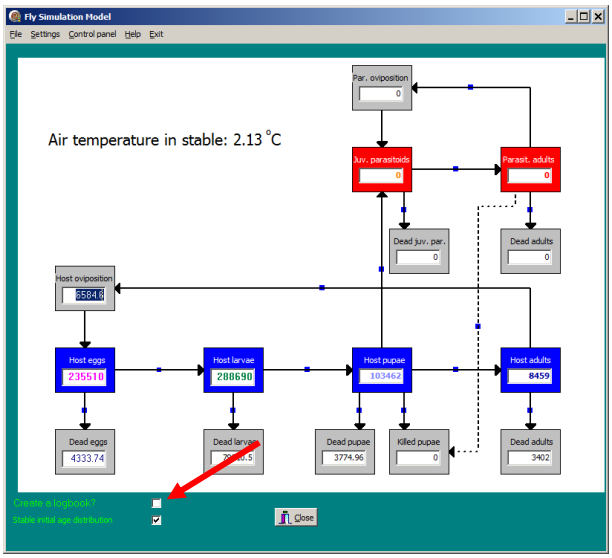

FIG. A3.23. THE LOGBOOK BOX TO BE TAGGED IF YOU WANT TO CREATE A LOGBOOK

When the simulation starts, a *logbook* appears. The run has automatically been given a number (the run ID). You can now enter your information associated with the run. When you close the logbook, the simulation starts. When the simulation ends, you will be asked whether your output from the simulation should be saved. Two files will be saved. Both have a name composed by the name of the scenario followed by the run ID. The two files differ with respect to file extensions, which are *.lbg for the logbook and *.sum. The former file contains your logbook (including some information about the initial stage of the system), while the latter contains summarized information about the system during the run. Both files are saved in a folder called “Output”, which is a subfolder in “FlySimulator”.

You can open the files by means of an editor program, for instance *NotePad*. The data in the *.sum files can easily be imported by a spreadsheet program (e.g. Excel) to allow for additional analyses or for drawing graphs.

A3.4.2. Reset numbering of logbook files

The files generated by the logbook get automatically a successive number. However, you can number the runs manually and you can reset the automatically numbering by clicking on the **Simulation** button on the *System* form or choose **Simulation** when you click on **Settings**. When the *Simulation Settings* form appears (Fig. A3.24), choose **Manual update of run number**. The box shows the number of the last run, so if you want to number the next run as #1, you should write 0 in the box. Then click on **Automatic update of run number**, the runs will be numbered successively starting with #1.

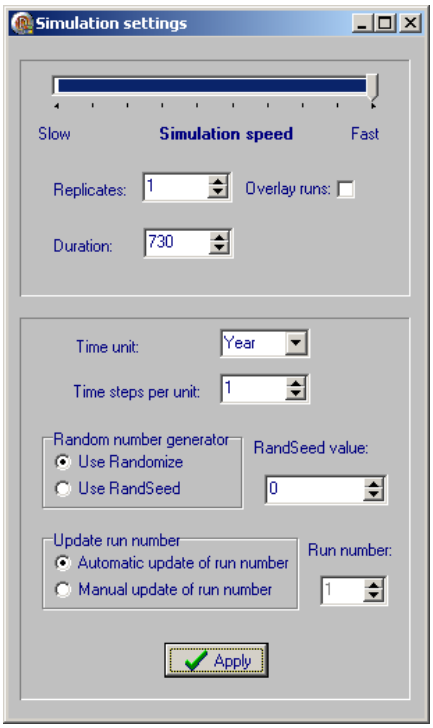

FIG. A3.24. THE *SIMULATION SETTINGS* FORM

A3.4.3. Change initial age distribution

If you remove the tag in the box called **Stable initial age distribution** on the *Fly Simulation Model* form, all individuals in a stage will be of age 0 when the simulation starts (instead of a stable age distribution). This feature is useful if you want to simulate a cohort in a life-table study.

A3.4.4. Change axes settings

You can modify the axes shown in the *System* form by clicking on the axis you want to change. For instance, you may use a transformation of the Y-axis or change the scale of the axis from being automatically scaled to being fixed.

A3.4.5. Change simulation speed

You can change the speed of a simulation by clicking on the **Simulation** button on the *System* form or click on **Settings** and choose **Simulation.** This makes the *Simulation Settings* form to appear (Fig. A3.24). The *Simulation* form is also used if you want to set the duration of a simulation, for instance if you want to simulate the host-parasitoid dynamics in a constant environment, but *not* if you use data from a specific stable as the duration of a simulation is determined by the data available.

Modelling Biological Control of Stable Flies by Means of Parasitoids

Stikfluer er et alvorligt problem i kreaturstalde. I konventionelle bedrifter bekæmpes fluerne (ofte utilstrækkeligt) med forskellige typer af biocider, og i økologiske bedrifter er det for tiden kun muligt med driftstekniske metoder at holde stikfluerne nede.

Der blev udviklet en simuleringsmodel, der beskriver det komplekse samspil mellem stikfluen, Stomoxys calcitrans, og dens naturlige fjende, snyltehvepsen, Spalangia cameroni. Modellen, 'The Fly Simulator', blev brugt til at analysere, hvordan snyltehvepsen bedst udnyttes i forbindelse med bekæmpelse af stikfluer.

Simuleringerne viser, at snyltehvepsene ikke er i stand til at overleve de lave staldtemperaturer om vinteren, og for at bekæmpelsen skal fungere, er det nødvendigt med løbende udsætninger af hvepse hen over sæsonen.

Simuleringer, der undersøgte effekten af udmugning viste, at problemer med stikfluer kan reduceres eller helt undgås ved at fjerne en stor del af gødningen gennem ofte og regelmæssige udmugninger.

Projektet har vist, at 'The Fly Simulator' vil kunne videreudvikles fra at være en strategisk model til en operationel (taktisk) model, som kan benyttes af den enkelte producent som en hjælp til at træffe de rigtige ad hoc beslutninger baseret på løbende informationer om situationen i stalden.

Environmental
Protection Agency
Strandgade 29
DK-1401 København K

www.mst.dk