*Biocontrol***News and Information** *2000 Vol. 21 No. 4* 105N – 116N

Review Article

The biology of *Toxorhynchites* mosquitoes and their potential as biocontrol agents

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Abstract

Toxorhynchites spp. mosquitoes are recognised as potential biological control agents of pest and vector species of mosquito. There have been many attempts to use them for this purpose since the beginning of the twentieth century, although with relatively low levels of success, which has been attributed to a lack of knowledge of the general biology of *Toxorhynchites* mosquitoes. Increasing resistance of vector mosquitoes to traditional chemical pesticides and the expansion of the ranges of these vector mosquitoes have made the search for alternative methods of mosquito control imperative. This review draws together the current knowledge of both the taxonomy and the general biology of *Toxorhynchites* mosquitoes and details previous attempts to use this group as biocontrol agents and in integrated control programmes. In addition, it makes recommendations for further study of this group in order to facilitate their successful utilization against vector mosquitoes.

Introduction

Mosquitoes (Dipt., Culicidae) are responsible for the transmission of the pathogens causing some of the most life-threatening and debilitating diseases of man, including malaria, yellow fever, dengue fever and filariasis. In many areas the incidence and geographical distribution of these diseases have expanded, largely as a result of decreased efficacy of vector-control programmes and subsequent increases in vector mosquito populations.

Mosquitoes are also becoming increasingly resistant to traditional chemical pesticides and there is growing concern about the potential health and environmental risks surrounding these products. Environmental protection agencies have banned or placed severe restrictions on the use of many pesticides which were formerly used in mosquito control programmes and there are now fewer adulticides available than there have been for the last 20 years (Rathburn, 1990). Furthermore, manufacturers themselves have withdrawn some insecticides due to the high cost of carrying out the additional tests now required by governments, in addition to the fact that the production of crop pesticides for the agricultural market is much more lucrative (Rathburn, 1990). It is likely, therefore, that mosquitoes will very quickly develop high levels of resistance to the remaining available adulticides, leading to concern among operational mosquito control personnel that effective insecticides may not be available in the near future (Kline, 1994). Hence, it is imperative that novel mosquito control methods are developed and put into general use as soon as possible.

One potential alternative approach to the use of chemical pesticides is the use of *Toxorhynchites* spp. mosquitoes as biological control agents of pest mosquitoes. This was suggested as early as 1911 by W. R. Colledge in an address to the Royal Society of Queensland and since this time there have been many attempts, some successful and some not. *Toxorhynchites* mosquitoes have an unusual life cycle in that they are not capable of blood feeding and, therefore, are not pests or vectors. In addition, their larvae are predatory on other mosquito larvae. In the development of any biological control strategy it is imperative that the biology and taxonomy of both the target species and the potential biological control agent are understood fully, which has not been the case for most *Toxorhynchites* species. Due to their lack of importance as pest species, their general biology and taxonomy have been largely neglected. Exceptions have been many isolated descriptions of particular aspects of the biology of some *Toxorhynchites* spp. mosquitoes, in addition to a small number of taxonomic studies.

This review draws together the current knowledge of the general biology and taxonomy of *Toxorhynchites* spp. mosquitoes. It also describes previous attempts to use these mosquitoes as biological control agents of vector mosquitoes and makes recommendations for further studies of this group.

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Table 1. Studies of the general biology of some *Toxorhynchites* species.

Species	Reference
T. amboinensis	Steffan *et al.* 1980
T. brevipalpis	Muspratt 1951; Corbet 1963; Corbet & Griffiths 1963; Lounibos 1979
T. kaimosa	Corbet & Griffiths 1963
T. rutilus rutilus	Jenkins & Carpenter 1946; Focks *et al.* 1977
T. rutilus septentrionalis	Jenkins & Carpenter 1946; Williams *et al*. 1961; Dodge 1964; Crans & Slaff 1977
T. splendens	Newkirk 1947; Breland 1949; Chan 1968; Furuzimo & Rudnick 1978
T. theobaldi	Rubio *et al*. 1980; Rubio & Ayesta 1984

Table 2. Studies of larval behaviour and population densities of *Toxorhynchites* species.

Species	Reference
T. amboinensis	Focks *et al.* 1981; Robert *et al.* 1983; Barber & Hirsch 1984; Russo 1986; Linley & Duzak 1989; Linley 1990
T. brevipalpis	Corbet & Griffiths 1963; Goma 1964; Sempala 1970, 1983; Trpis 1972, 1973; Lounibos 1979; Vongtangswad & Trpis 1980; Lamb & Smith 1980; O'Flynn & Craig 1982; McIver & Siemicki 1982; McIver & Beech 1986; Robert *et al*. 1983; Schuler & Beier 1983; Russo 1986; Linley 1990; Linley & Duzak 1989
T. haemorrhoidalis	Lounibos *et al*. 1987
T. kaimosa	Goma 1964; Sempala 1983
T. moctezuma	Chadee 1985; Sherratt & Tikasingh 1989; Rawlins & Ragoonansingh 1990; Chadee & Small 1991; Rawlins *et al*. 1991; Tikasingh 1992; Tikasingh & Eustace 1992
T. rutilus	Focks *et al.* 1978, 1980; Trimble & Smith 1978, 1979; Lamb & Smith 1980; Padgett & Focks 1980, 1981; Russo 1983, 1986; Schuler & Beier 1983; Bradshaw & Holzapfel 1984; Frank *et al*. 1984; Lounibos 1985; Lounibos *et al*. 1993; Hubbard *et al*. 1988
T. splendens	Newkirk 1947; Russo 1986; Vongtangswad *et al*. 1983; Linley & Duzak 1989; Toma & Miyagi 1992; Amalraj & Das 1996
T. theobaldi	Russo 1986
T. towadensis (Matsumura)	Yasuda 1996

General Biology of *Toxorhynchites* Mosquitoes

The biology of *Toxorhynchites* species has been reviewed by several authors since Colledge's address on the biology of *Toxorhynchites speciosus* Skuse (Colledge, 1911). These reviews have dealt with specific groups of *Toxorhynchites* mosquitoes (MacDonald, 1958; Horsfall, 1972), the wider biology of the *Toxorhynchites* group (Steffan & Evenhuis, 1981), in addition to their potential as biological control agents (Focks, 1982; Gerberg, 1985). A small number of species with potential as biological control agents has been considered more fully (Table 1).

Eggs

The eggs of *Toxorhynchites* spp. mosquitoes are white or yellow, oval and water repellent and are found either floating on top of the water surface or just below it. The incubation period is 40-60 hours and is temperature dependent. Egg viability is 57-100% and decreases with the age of the female in some species (Steffan & Evenhuis, 1981). A description of the general structure of *Toxorhynchites* eggs is included in Sahlen (1996).

Larvae

All instars of *Toxorhynchites* spp. larvae are predatory. They are traditionally thought to feed on mosquito larvae; most commonly approximately the same size as themselves, although it is thought that they will also feed on larvae up to twice their size. They will also take almost any type of moving prey and in the absence of live prey, they will feed on detritus (Steffan & Evenhuis, 1981).

The feeding rate and total prey consumption during larval development depend on a number of factors, including container size, prey size, prey type, water temperature and light level. During its development, one *Toxorhynchites* spp. larva requires approximately 5000 first-instar prey larvae and 300 fourth-instar prey larvae (Steffan & Evenhuis, 1981; Focks, 1982). The larvae of a number of *Toxorhynchites* species have been described and their predatory behaviour studied. In particular, descriptions of the searching behaviour and population densities of *Toxorhynchites* spp. larvae in relation to prey larva populations have been published for a number of species (Table 2).

Prepupal killing behaviour has been observed in the larvae of two *Toxorhynchites* species; *Toxorhynchites brevipalpis* Theobald

(Corbet & Griffiths, 1963) and *Toxorhynchites amboinensis* (Doleschall) (Taylor, 1989). Just before pupation, fourth-instar larvae kill but do not consume prey larvae. The most widely accepted theory to explain this behaviour is that any other potential predators in the same aquatic environment are killed before the *Toxorhynchites* spp. larvae become vulnerable pupae (Corbet & Griffiths, 1963). Russo & Westbrook (1986) hypothesized that this behaviour is analogous to changes in feeding behaviour at the same stage in other insects and attempted to show that it was similarly governed by increases in ecdysteroid levels.

The larvae of many species of *Toxorhynchites*, in addition to being predatory, are cannibalistic in all instars. The degree of cannibalism displayed depends on prey density and behaviour, size of prey relative to *Toxorhynchites* spp. larvae and the number of hiding places in the container (Focks, 1982). The cannibalistic behaviour of a few *Toxorhynchites* species has been described in detail, along with the effects it has on the potential of the species as a biological control agent. These species included *T. amboinensis* (Focks *et al.*, 1981; Linley, 1988; Linley & Duzak, 1989; Annis *et al.*, 1990; Horio *et al.*, 1990), *T. brevipalpis* (Muspratt, 1951; Sempala, 1983; Linley & Duzak, 1989), *Toxorhynchites kaimosa* van Someren (Sempala, 1983), *Toxorhynchites longgianeolata* Macquart (Farghal, 1983) and *Toxorhynchites moctezuma* (Dyar & Knab) (Sherratt *et al.*, 1999). Descriptions of egg cannibalism have also been made for *T. moctezuma* (Chadee & Small, 1991), *T. amboinensis* and *Toxorhynchites rutilus* (Coquillett) (Linley & Darling, 1993).

The duration of larval development in *Toxorhynchites* spp. mosquitoes varies from 1-91 days, depending on species, temperature and prey density (Steffan & Evenhuis, 1981), followed by a pupal period of 3-12 days, which is dependent mainly on temperature (Steffan & Evenhuis, 1981). Particular aspects of the larval development of the following *Toxorhynchites* species have been studied: *Toxorhynchites splendens* (Wiedemann) (Jones, 1993), *T. rutilus* (Trimble & Smith, 1978, 1979; Trimble & Lund, 1983; Bradshaw & Holzapfel, 1984), *Toxorhynchites theobaldi* (Dyar & Knab) (Rubio *et al.*, 1980), *T. amboinensis* (Robert *et al.*, 1983), *T. brevipalpis* (Sempala, 1970; Trpis, 1972, 1979; Robert *et al.*, 1983) and *Toxorhynchites haemorrhoidalis* Ficalbi (Lounibos *et al.*, 1987).

At least one species, *Toxorhynchites rutilus septentrionalis* (Dyar & Knab), is capable of diapausing. This occurs only in fourth-instar larvae and is photoperiodically controlled. It allows the larvae to survive temperatures as low as 7ºC (Steffan & Evenhuis, 1981) and hence, to survive as far north as New Jersey, USA, where *T. rutilus septentrionalis* larval development varies between diapausing populations in the north and non-diapausing populations in the south (Trimble & Smith, 1978, 1979).

Adults

There have been very few observations of *Toxorhynchites* spp. adults in the wild. This is particularly true for the males since most studies, both in the laboratory and in the field, have involved observations of female oviposition behaviour. Eclosion in *Toxorhynchites* spp. is not synchronized, it occurs during daylight and, depending on the species, can be protogynous or protandrous. For example, *Toxorhynchites brevipalpis conradti* Grünberg is protogynous (Corbet, 1963), whereas *T. rutilus septentrionalis* is protandrous (Crans & Slaff, 1977). Adult female *Toxorhynchites* spp. mosquitoes are non-haematophagous, feeding on only nectar and other plant derived sugar sources. There have, however, been only a small number of observations to confirm this. These include records of *T. moctezuma* (as *T. trinidadensis*) feeding on the flowers of the Christmas bush (*Chromolaena odorata*; Asteraceae) and black sage (*Salvia mellifera*; Labiatae) (Urich, 1913), *Toxorhynchites haemorrhoidalis superbus* (Dyar & Knab) feeding on *Borreria verticilata* (Rubiaceae) flowers (Heinemann *et al.*, 1980) and *T. rutilus septentrionalis* feeding on the nectar of *Hydrangea* (*Hydrangea macrophylla*; Hydrangeaceae) flowers (Williams *et al.*, 1961) and on the sap of the black oak tree (*Quercus velutina*; Fagaceae) (Nasci, 1986).

Very little is known about the adult dispersal and population sizes of *Toxorhynchites* spp. in the wild. A mark-release-recapture study of *T. brevipalpis* in a tyre dump in Dar es Salaam, Tanzania estimated the population to be 1292 females and 2268 males per hectare (Trpis, 1973). A method devised for ^{32}P labelling *Toxorhynchites rutilus rutilus* (Coquillett) in the field had the potential of being used to track the dispersal of released adults, in addition to the dispersal of eggs from released females (since ^{32}P is also passed on to the eggs) (Smittle & Focks, 1986). To date, however, it has not been trialed in the field.

The oviposition behaviour of female *Toxorhynchites* spp. mosquitoes is relatively well documented. The fullest description of this is a study by Linley (1987a) of the oviposition flight of *T. amboinensis.* The females rarely land on the water surface to oviposit; instead they perform an oviposition flight which consists of six to 43 elliptical loops, with the egg being released on the final loop. The diel rhythm of oviposition in *Toxorhynchites* spp. mosquitoes varies. For example, in the wild, peak oviposition frequency in *T. moctezuma* occurs between 1200 h and 1700 h (Chadee *et al.*, 1987; Jordan & Hubbard, 1991a) compared with the major oviposition peak for *T. amboinensis* in the laboratory, which occurred between 1200 h and 1500 h, with a smaller peak between 0800 h and 1000 h (Linley, 1987b). The reasons for these patterns of diel periodicity are unclear but may be related to humidity (Jordan & Hubbard, 1991a) and rainfall patterns. *Toxorhynchites* spp. mosquitoes oviposit during the rainy season but not during the dry season. They survive the dry season as fourth-instar larvae, then complete their juvenile development and eclose at the beginning of the rainy season as the prey density increases. For example, *T. rutilus* ecloses approximately two weeks after the beginning of the rainy season (Bradshaw & Holzapfel, 1984).

Distribution and Habitat of *Toxorhynchites* Mosquitoes

Toxorhynchites mosquitoes inhabit most of the tropical regions of the world. Edwards (1932) described the group as 'tropicopolitan' and Horsfall (1972) gave the distribution of the group as extending to 40ºN and south to the edge of the southern tropics. They also inhabit some subtropical and temperate regions. For example, *Toxorhynchites christophi* (Portschinsky) has been found in the Amur Valley in Russia (54°N) (Shamrai & Gutsevich, 1974) and *T. rutilus septentrionalis* is found up to 45°N in Canada (Parker, 1977). Members of the *Lynchiella* subgenus have also been reported as far north in the USA as New Jersey and as far west as Kansas and Texas (Jenkins & Carpenter, 1946). Stone *et al.* (1959) detailed the regions inhabited by the three subgenera of the Toxorhynchitinae subfamily (Table 3).

Tropical and subtropical forests are the main vegetation types within which *Toxorhynchites* spp. mosquitoes are found (Muspratt, 1951), although they also inhabit coastal palm belts; for example *T. splendens* is found in this habitat in India, Sri Lanka, Malaysia, Thailand, Papua New Guinea and the Philippines (MacDonald, 1958). It was suggested that this wide distribution may have been facilitated by transportation of the larvae to new sites in water storage containers on ships.

Table 3. Regions inhabited by the three subgenera of Toxorhynchitinae (Stone *et al*., 1959).

Subgenus	Region/country
Ankylorhynchus (Lutz)	Argentina, Brazil, Bolivia
Lynchiella (Lahille)	South America: Argentina, Paraguay, Brazil, Peru, Ecuador, Surinam, Guyana, French Guiana, Venezuela, Colombia
	Central America: Panama, Costa Rica, Nicaragua, Honduras, El Salvador, Guatemala, Mexico
	Caribbean: Greater Antilles (Puerto Rico, Haiti, Cuba), Lesser Antilles, Trinidad
	Southeast USA (Florida, Georgia, South Carolina).
Toxorhynchites Theobald	Australasian Region: Australia, Papua New Guinea (including Bismarck Archipelago), Tuvalu, Hawaii
	Oriental Region: eastern Siberia, western Himalayas, Indochina, including Japan (including Ryukyu Islands), Taiwan, Philippines, Malaysia (Malaya) Singapore, Indonesia (Sumatra, Java, Maluku, Sulawesi, Borneo), Thailand, India, Sri Lanka
	Ethiopian Region: Gambia, Sierra Leone, Liberia, Ghana, Nigeria, Cameroon, Gabon, Uganda, Kenya, Tanzania (including Zanzibar), Democratic Republic of Congo, Malawi, Mozambique, South Africa (Transvaal, Natal, Cape Province), Madagascar

Table 4. Regional reviews of the taxonomy of *Toxorhynchites* species.

Region	Review
New World	Dyar 1928; Vargas 1953a, b; Lima *et al*. 1962
Central and North America	Howard *et al*. 1917
Neotropical Region	Lane 1953
Caribbean Region	Belkin *et al*. 1970
Afrotropical Region	Hopkins 1952; Service 1990
India	Barraud 1934
South Pacific	Belkin 1962

Classification of *Toxorhynchites* Mosquitoes and Keys to the Group

Partly due to their unimportance as pest species, the taxonomy of *Toxorhynchites* spp. mosquitoes has been largely neglected. *Toxorhynchites* were first recognised as a distinct group in 1827 when Robineau-Desvoidy proposed that they be known as *Megarhinus* (Robineau-Desvoidy, 1827). Since this work there have been only two taxonomic revisions of the whole group. These are the works of Theobald (1901) and Edwards (1932) on Culicidae, in which the *Toxorhynchites* (as *Megarhinus*) were included.

Edwards (1932) divided the 52 species known at the time into three groups based on the differences in the maxillary palps of the adult females. Group A, *Megarhinus*, was composed of 20 Neotropical species; Group B, *Ankylorhynchus*, contained two Brazilian species and Group C, *Toxorhynchites*, was composed of 30 species from the Old World. There have, however, been many regional studies of the taxonomy of *Toxorhynchites* mosquitoes (Table 4), although the taxonomy of the *Toxorhynchites* species of the New World is still thought to need clarification (Steffan, 1975). More recently, descriptions of type specimens in museums have been produced, including those held at the United States National Museum of Natural History (Steffan, 1980) and those held at the British Museum (Natural History) and Oxford University (Steffan & White, 1981). A key to and descriptions of the Afrotropical Toxorhynchitinae have also been produced recently (Service, 1990).

Descriptions of new *Toxorhynchites* species or species new to a particular area have been added to the literature as they have been identified. Recent examples include descriptions of *Toxorhynchites yaeyamae* Bohart from Japan (Sâto & Arita, 1968), *Toxorhynchites bengalensis* Rosenberg & Evenhuis from Bangladesh (Rosenberg & Evenhuis, 1985), *Toxorhynchites auranticauda* Lane from Indonesia (Lane, 1992), *T. rutilus septentrionalis* from Rhode Island (Lawson *et al.*, 1994) and *Toxorhynchites macaensis* Ribeiro from Macau (Ribeiro, 1997).

Due to the fragmented nature of the taxonomy of the *Toxorhynchites* group, the same species has often been described more than once but has been named differently on each occasion. Also, the interspecific relationships of *Toxorhynchites* spp. mosquitoes are unclear and most of the species are very similar to each other. This has resulted in serious implications for biological control attempts using *Toxorhynchites* species. For example, the species introduced into Hawaii as *T. splendens* was later found to be *T. amboinensis* and this error was repeated when *T. amboinensis* was introduced into other Pacific Islands as a biological control agent (Steffan, 1975).

Stone *et al*. (1959) and Stone (1961, 1963, 1967, 1970) in their Synoptic Catalog of the Mosquitoes of the World and its supplements listed 66 *Toxorhynchites* species, divided into three

subgenera: *Ankylorynchus*, *Lynchiella* and *Toxorhynchites*. However, by 1975 only 53% of these species were known in all stages and the eggs of only five species had been described (Steffan, 1975). There have been some more recent descriptions to add to this, particularly of the eggs, including those of *T. splendens* (Mattingly, 1969; Linley & Seabury, 1990), *T. brevipalpis* (Lamb & Smith, 1980), *T. rutilus* (Lamb & Smith, 1980; Linley 1989), *T. amboinensis* (Steffan *et al.*, 1980; Russo & Westbrook, 1986; Linley, 1989; Linley & Seabury, 1990) and *T. moctezuma* (Chadee *et al.*, 1987). In addition, the pupa of *T. rutilus septentrionalis* has been described (Steffan & Evenhuis, 1980)

The most recent classification of *Toxorhynchites* spp. dealt with the *Toxorhynchites* group regionally; for example, the first section covered the Australasian, east Palaearctic, and Oriental species-groups (Steffan & Evenhuis, 1985). The authors tried to clarify the complex interspecific relationships in the group by taking into account both intuitive taxonomic methods and numerical phylogenetic studies. They also included keys to the *Toxorhynchites* species found in each region.

Biological Control of Pest and Vector Mosquitoes

The increasing problem of how to control pest and vector mosquito populations effectively was recognised in 1982 when the World Health Organization (WHO) Expert Committee on Vector Biology and Control met in Geneva. The then Director of the Division of Vector Biology and Control, Dr N. G. Gratz, stated that although the use of residual pesticides had provided a cheap and simple way to control vectors of disease, the increase in pesticide resistance and the environmental damage caused by these pesticides had rendered their long-term use unfeasible (WHO, 1982). The 23rd World Health Assembly in 1970 had previously recommended the development of alternative methods of vector control (World Health Assembly, 1970).

Alternative methods of mosquito control had been investigated and evaluated even before this time. In 1950 H. H. James reported to the Quebec Society for the Protection of Plants on a number of possible biocontrol agents of mosquito larvae (James, 1950). The natural predators that they identified as possible biocontrol agents were dragonfly nymphs, damselfly nymphs, dytiscid beetles, phantom midge larvae, corixid water bugs and two species of stickleback fish. Ducks have also been identified as natural predators of mosquitoes, for example the Australian whistling tree duck (*Dendrocygna arcauta*) and the black duck (*Anas superciliosa*) feed on at least two species of *Culex* mosquito larvae (Marks & Lavery, 1959). Ramoska & Sweet (1981) have also observed a spider (*Agelenopsis naevia* (Walckenaer) (Araneae, Agelenidae)) which is a natural predator of adult mosquitoes ovipositing into discarded tyres. Mosquito fish (*Gambusia affinis*) have been introduced to storm drains in California to control *Culex quinquefasciatus* Say (Mulligan *et al.*, 1983). *Culex* larvae have also been controlled using the planarian *Dugesia dorotocephala* (Ali & Mulla, 1983) and in New Zealand, *Aedes australis* (Erichson) mosquitoes have been successfully controlled in experimental pools by the fungus *Coelomomyces opifexi* Pillai, which has a copepod as its intermediate host (Pillai, 1985).

The bacterium *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) has been used widely and successfully as a biological control agent against mosquito larvae. For example, it was lethal to eight mosquito species, including *Aedes aegypti* (L.) and *Culex quinquefasciatus* in the laboratory and was effective in the field against *Culex tarsalis* Coquillett (Mulla *et al.*, 1982). In addition, it successfully controlled *Culex sitiens* Wiedemann, a vector of Ross River Virus in Fiji (Pillai, 1985). The related *Bacillus sphaericus* has also been used against mosquito larvae. For example, in a field trial in Recife, Brazil, it successfully controlled *C. quinquefasciatus* (Regis *et al.*, 1995). There is, however, evidence that *C. quinquefasciatus* is capable of developing resistance to *B. sphaericus* (Rodcharoen & Mulla, 1994) and at least one other species of mosquito, *Aedes albopictus* (Skuse), is susceptible to *Bti* but not to *B. sphaericus* (Ali *et al.*, 1995). In addition to resistance, another major problem of the use of *Bacillus* biolarvicides against mosquitoes is that their toxic proteins are not sufficiently residual in the environment, although there have been attempts to overcome this problem, including genetically engineering the micro-organisms on which mosquito larvae feed to express *Bti* and *B. sphaericus* toxic proteins (Orduz *et al.*, 1995).

There is one main disadvantage in using any of the above biological control agents: they have to be manually introduced to the majority of pest and vector larval habitats to yield adequate control, which requires regular maintenance. *Toxorhynchites* spp. mosquitoes offer an alternative form of biological control. Their predatory larvae often feed upon the larvae of vector species of mosquito and hence, *Toxorhynchites* spp. adult females seek out these same aquatic habitats to lay their eggs. An additional tool in this respect might be the exploitation of oviposition cues for *Toxorhynchites* species. They could, for example, be used to encourage released adult female *Toxorhynchites* spp. mosquitoes to remain in the target area, in addition to increasing the efficiency of any release programme since both predator and prey may be attracted to the same oviposition sites. Although oviposition aggregation cues or pheromones have yet to be used on a regular basis with *Toxorhynchites* spp., potential oviposition cues for *T. moctezuma* and *T. amboinensis* have been identified in electroantennogram studies and oviposition bioassays. Electroantennograms were recorded for seven compounds found commonly in water containing decaying leaves and known to be attractive to other mosquito species: 4-methylcyclohexanol, phenol, indole, 3-methylindole, *m*-cresol, *o*-cresol and *p*-cresol (Collins & Blackwell, 1998). In laboratory oviposition bioassays 4-methylcyclohexanol, 3-methylindole, *m*-cresol, *o*-cresol and *p*-cresol were found to act as oviposition attractants for both of these species (Collins & Blackwell, in press). For other species, oviposition aggregation cues have been used successfully to monitor pest mosquito populations of *A. aegypti* (Reiter *et al.*, 1991) and there has been a successful field trial in Kenya using oviposition pheromone combined with an insect-growth regulator to control *C. quinquefasciatus* (Otieno *et al.*, 1988).

Biological Control of Pest and Vector Mosquitoes Using *Toxorhynchites* Species

Toxorhynchites spp. mosquitoes are potentially ideal biological control agents, as highlighted by Trimble (1983): the adults do not blood feed and therefore cannot themselves act as vectors of disease; the larvae are predatory on other mosquito larvae and show ‘prepupal killing’ behaviour (before pupation they kill but do not consume large numbers of potential prey) and in addition, female *Toxorhynchites* spp. oviposit into pools of water which are not accessible to chemical control methods. However, biological control using *Toxorhynchites* spp. mosquitoes has not always been successful, often because introduced *Toxorhynchites* spp. populations have not always become established and, even if they have, established *Toxorhynchites* spp. populations have frequently failed to give an adequate level of control of pest mosquitoes. One reason for this failure is that there is a delay between the pest mosquito and *Toxorhynchites* spp. population increases, since *Toxorhynchites* spp. mosquitoes have a generation time which is approximately three times longer than that of their prey; resulting

in a high peak in the pest mosquito population before it is able to be challenged by the *Toxorhynchites* spp. (Trpis, 1973; Lounibos, 1979; Service, 1983). Additional failures in control can also occur if the established *Toxorhynchites* spp. populations inhabit and oviposit into different aquatic habitats from the target species (Focks, 1982; Service, 1983).

The Pacific islands have been popular test sites for introductions of *Toxorhynchites* spp. as biocontrol agents, with the first attempts carried out on Hawaii in 1929 (Swezey, 1930; Pemberton, 1931), followed by releases of *T. brevipalpis* from Africa in 1950 (Bonnet & Hu, 1951), *T. theobaldi* from Panama in 1953, and *T. amboinensis* (as *T. splendens*) from Manila in 1953 (Steffan, 1975). Integration of *Toxorhynchites* spp. into filariasis-control programmes on the Hawaiian Islands was the aim of the introduction of *T. amboinensis* (as *T. splendens*) in 1955, although with relatively little success (Hu, 1955). For example, by 1963 it was clear that although *T. amboinensis* had become established in Hawaii it did not adequately control its target organism, *A. albopictus* (Nakagawa, 1963) and on the island of Oahu, onto which both *T. brevipalpis* and *T. amboinensis* had been introduced, *T. amboinensis* has displaced *T. brevipalpis* (Steffan, 1970). Greater success was, however, recorded in other areas. For example, following the introduction of *T. amboinensis* (as *T. splendens*) and *T. brevipalpis* onto American Samoa in 1955 to control the filariasis vector *Aedes polynesiensis* Marks (Peterson, 1956); it was reported in 1978 that *T. amboinensis* had become established and was effectively controlling the target mosquito (Engber *et al.*, 1978).

Problems relating to the mis-match of oviposition sites between the target and control species were highlighted when *T. splendens* was introduced onto Fiji in 1934 to control *A. polynesiensis* (Paine, 1934), followed shortly by *Toxorhynchites inornatus* (Walker) (Lever, 1938). *Toxorhynchites splendens* became established but did not provide adequate control of *A. polynesiensis*, since their oviposition sites did not coincide (Toohey *et al.*, 1985). An attempt was made to overcome this problem in 1979 with the introduction of *T. amboinensis*, and by 1985 it was clear that this had been successful in controlling the *A. polynesiensis* population (Toohey *et al.*, 1985). The introduction of *T. amboinensis* larvae was less successful in 1987 when introduced into domestic water storage containers on Java in an attempt to control *A. aegypti* and *A. albopictus*, vectors of the dengue virus. Little control was achieved due to the large number of untreated natural containers in which the target mosquitoes were breeding (Annis *et al.*, 1990).

Attempts to control pest and vector mosquitoes using *Toxorhynchites* spp. mosquitoes have been made in many regions of the world. For example, *T. splendens* has been used successfully in Pudapet, a coastal village near Pondicherry on the Coromandel coast of India, where there were significant reductions in the numbers of *A. aegypti*, *Armigeres subalbatus* (Coquillett) and *C. quinquefasciatus* breeding in domestic water containers six months after treatment began (Panicker & Geetha Bai, 1983). Second-instar *T. splendens* larvae were also used successfully to suppress *Aedes aegypti* and *A. albopictus* in domestic water containers in Malaysia (Chuah & Yap, 1984). However, when first-instar *T. splendens* were used similarly against the same target mosquitoes in Jakarta, Indonesia in 1987, the *A. aegypti* population was not affected, probably because the first-instar larvae were unable to withstand starvation (Annis *et al.*, 1989). Fourth-instar *T. splendens*, however, were used successfully to reduce the *A. aegypti* population on Sa-Med Island, Thailand in 1979 (Vongtangswad *et al.*, 1983).

There have been a number of studies of the suitability of *Toxorhynchites* spp. mosquitoes as biological control agents in Africa. For example, Muspratt (1951) on *T. brevipalpis*, Corbet (1963, 1964) on *T. brevipalpis conradti* in Uganda, and Sempala (1983) on *T. brevipalpis conradti* and *T. kaimosa*, also in Uganda.

Toxorhynchites spp. have also been considered as possible biological control agents in the USA. For example, Focks *et al.* (1979) released adult *T. rutilus rutilus* into an area of Gainsville, Florida to control *A. aegypti* and although the adults moved out of the urban area into the forest and deposited some of their eggs into non-target oviposition sites, there were enough ovipositions into target sites to give adequate levels of *A. aegypti* control. In an urban area of New Orleans, Louisiana, the introduction of first-instar *T. rutilus rutilus* larvae resulted in a 74% reduction in the levels of *A. aegypti* and *C. quinquefasciatus* (Focks *et al.*, 1982). Another study using *T. rutilus rutilus* in Louisiana (Focks *et al.*, 1983) was less successful, since oviposition occurred more often into tree holes than into the artificial containers in which the *A. aegypti* were breeding. Bailey *et al.* (1983), however, reduced *A. aegypti* populations by 50% in tyre dumps in Jacksonville, Florida by introducing *T. rutilus rutilus* larvae to the tyres. In a later study in New Orleans, *T. amboinensis* releases were combined with ultra-low volume (ULV) malathion spraying, resulting in a 96% reduction in the *A. aegypti* density, whereas treatment with ULV malathion alone reduced the *A. aegypti* by only 29% (Focks *et al.*, 1986).

Over the last twenty years, there have also been attempts to use *Toxorhynchites* spp. mosquitoes as biological control agents in the Caribbean islands. For example, on the island of St. Maarten, *T. brevipalpis* larvae released into domestic water containers reduced the house index of *A. aegypti* to zero (Gerberg & Visser, 1978) and in 1985 it was reported that a native species in Trinidad and Tobago, *T. moctezuma*, was potentially a good biological control agent for *A. aegypti* since it tends to oviposit in the same places as the target mosquito (Chadee, 1985). A study of the effectiveness of a single introduction of *T. moctezuma* larvae on Union Island resulted in a reduction in the number of adult *A. aegypti*, although there was some doubt as to whether this reduction was actually due to the introduction of the *T. moctezuma* larvae (Rawlins *et al.*, 1991). In a laboratory study it was found that the introduction of between five and ten *T. moctezuma* larvae to each water container reduced the *A. aegypti* population to 0-0.6% for 16 weeks (Tikasingh, 1992), and sequential releases of *T. moctezuma* larvae in a village on Union Island resulted in a significant reduction in the *A. aegypti* population (Tikasingh & Eustace, 1992).

It can be concluded from the examples above that *Toxorhynchites* spp. mosquitoes do not all oviposit into *A. aegypti* infested containers in urban environments. Also, that higher levels of control are produced if *Toxorhynchites* spp. larvae are introduced rather than adults. This led Gerberg (1985) to recommend sequential introduction of *Toxorhynchites* spp. eggs and larvae to infested containers rather than releasing adults. In addition to this, Jones (1993) recommended the introduction of fourth-instar *Toxorhynchites* spp. larvae to containers before the target mosquito population increases. The overall belief of workers is that, although *Toxorhynchites* spp. mosquitoes are unable to control target species instantly, they could provide long term control once the introduced population has become established (Focks, 1982).

Integrated Mosquito Control Using *Toxorhynchites* Mosquitoes, *Bacillus* Toxins and Traditional Chemical Pesticides

Recently, there have been investigations into the suitability of *Toxorhynchites* spp. mosquitoes for use in integrated pest management (IPM) programmes, both with *Bacillus* toxins and

with traditional chemical insecticides. Problems have arisen in combining *Bti* and *B. sphaericus* with some *Toxorhynchites* spp., in that effective *Bti* doses may be lethal to the *Toxorhynchites* larvae. For example, in laboratory studies, *Bti* effective doses for the control of *A. aegypti* larvae were lethal to first and second-instar *T. rutilus rutilus*, although not to older instars of the same species (Lacey & Dame, 1982). *Bacillus sphaericus* toxin, although only moderately effective against most *Aedes* species, does control *Culex* and *Anopheles* species. Furthermore, although *B. sphaericus* toxin was lethal to *T. rutilus rutilus*, it was not to *T. theobaldi*, *T. brevipalpis* and *T. amboinensis* (Lacey, 1983).

In a laboratory study of the susceptibility of *T. amboinensis* to a variety of insecticides used for control of *A. aegypti*, the vector mosquito larvae were significantly more susceptible than *T. amboinensis* to naled and chlorpyrifos. Most insecticides, however, were lethal to both predator and prey mosquitoes. Only a few insecticides appear promising for combination with *Toxorhynchites* spp. larvae. For example, the organophosphate temephos has a sufficiently low toxicity to *T. moctezuma* larvae to be useful in IPM programmes (Rawlins & Ragoonansingh, 1990) and naled was the least toxic organophosphate compound to *T. splendens* larvae (Tietze *et al.*, 1993). Malathion also appears promising; releases of *T. splendens* along with ULV applications of malathion have been used in four cities in Florida. This combination produced better levels of control of *A. aegypti* and other pest mosquitoes than the use of ULV malathion applications alone (Schreiber & Jones, 1994). An alternative suggestion has been the sequential alternation of insecticides with *Toxorhynchites* spp. releases, with the aim of reducing insecticide selection pressure on vector mosquito populations (Djam & Focks, 1983).

Conclusions

It is evident from attempts to use *Toxorhynchites* spp. mosquitoes as biological control agents that it is essential that the chosen species can become established in the area in which control is required, and that it will oviposit into the same aquatic environments as the target mosquito species. *Toxorhynchites* spp. have been used successfully to control vector mosquito species, although the fact that these cases have been successful is due, at least in part, to chance, since very little was known of their biology and taxonomy at the time.

The modern techniques of molecular biology should be used to clarify the complex relationships between species and to define species complexes which cannot be defined using traditional taxonomy. Although much is now known of the general biology of some *Toxorhynchites* species, there are still large areas in which a greater understanding of their biology would be advantageous in the successful choice of particular *Toxorhynchites* species as biocontrol agents. For example, it is necessary to understand fully both the population dynamics of *Toxorhynchites* spp. in their natural habitat, and the predator-prey relationships between *Toxorhynchites* spp. larvae and their prey. Most importantly, there is a great need for studies of the oviposition site choices and oviposition cues of a variety of *Toxorhynchites* species in their natural habitat. These oviposition cues could then be matched to the oviposition cues of target species and increase the efficiency of adult release programmes. They could also be exploited to attract both *Toxorhynchites* spp. and target species to oviposition traps.

Acknowledgements

This work was funded by a Natural Environment Research Council Postgraduate Studentship.

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