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Butyric acid, putrescine and their mixture as potential fly attractants: Complexation, thermal analysis and volatility characterisation

Franco Pretorius, Elizabeth L. du Toit, Isbé van der Westhuizen, Maria T. Atanasova, Walter W. Focke*

Department of Chemical Engineering, University of Pretoria, Private Bag X20, Hatfield 0028, Pretoria, South Africa

A B S T R A C T

Mixtures of butyric acid and putrescine form a crystalline amine carboxylate complex in a 2:1 molar ratio irrespective of the initial molar component mixture ratio. This was confirmed through X-ray diffraction, FTIR spectroscopy and elemental analyses. Thermogravimetric analyses proved this complex to be significantly less volatile than the pure constituent components. In thermogravimetric temperature scans, a 90% mass loss of the complex was achieved at 143.4 °C while the same level was achieved at 68.1 °C for both the pure putrescine and butyric acid. A gas permeability parameter $\Phi_A = M_A D_A P_A / RT$ (g m^{-1}s^{-1}) was developed to quantitatively describe the volatility of the respective components through a stagnant gas layer. This parameter is a function of only material properties and temperature and adequately described the observed mass loss behaviour of pure components as well as the newly formed complex. In a preliminary field trial, mixtures of putrescine and butyric acid were less effective than pure putrescine for attracting flies. This can be attributed to the lower volatility of the putrescine/butyric acid complex and the subsequent suppression of the release rate of the active compounds.

1. Introduction

Carrion flies are global pests which transmit various antibiotic-resistant zoonotic diseases [1]. They are now considered to be some of the most insecticide-resistant species [2]. An attract-and-kill approach with species-specific attractants placed in traps could act as a more environmentally friendly alternative to indiscriminate pesticide use. Flies of the families Calliphoridae (blow flies), Oestridae (bot flies) and Sarcophagidae (flesh flies), among others, are strongly attracted to rotting meat [3]. Putrefying flesh, whether from dead animals or open wounds, emits a pungent smell. Putrescine is one of the compounds responsible for this foul odour which is released by bacterial metabolism of the substrate [4]. Butyric acid is a pungent, short-chain fatty acid that contributes to the rancid aroma of overripe fruit. The fruit fly families Drosophilidae and Tephritidae, and even house flies [5], find this compound attractive [6].

Insect traps are used to monitor and even control insect infestations [7]. They function by releasing an odour which is attractive to a flying insect. Unsurprisingly, slow-release fly attractants have included *n*-butanoic acid (butyric acid) and/or 1,4-diaminobutane (putrescine) as formulation ingredients [8,9].

Butyric acid is a highly volatile compound which evaporates quickly in outdoor applications. Furthermore, in fly attractant formulations putrescine is usually applied in combination with trimethylamine (which is a vapour at room temperature) as the hydrochloric acid salts [5]. The question arose whether it would be possible to replace the hydrochloric acid with butyric acid. The hypothesis was that this would similarly reduce the volatility of the latter. Ideally, this could lead to a hydrochloric acid-free, longer lasting lure formulation provided that the combination retains its attractiveness to flies. Therefore, this communication reports on an exploratory investigation of the chemical interaction between butyric acid and putrescine, thermal analysis of the volatility of these combinations, and also on a preliminary field trial that focused on trapping flies in an orange orchard.

2. Materials and methods

2.1. Materials

Butanoic acid (butyric acid) [CAS: 107-92-6], 1,4-diaminobutane (putrescine) [CAS: 110-60-1], and benzoic acid [CAS: 65-85-0] were obtained from Merck (Pty) Ltd., Lethabong, South Africa. The supplier

indicated that the purity of these compounds exceeded 99 %. Therefore, they were used as received, i.e. without further purification. Capsules containing Questlure protein hydrolysate, a commercial fly attractant produced by River Bioscience, Gqeberha, was supplied by Wenkem SA, Irene, South Africa.

2.2. Methods

2.2.1. Sample preparation

A range of butyric acid–putrescine mixtures were prepared. The mole ratio was varied from 1:4 to 4:1. The reaction between butyric acid and putrescine was found to be extremely exothermic. Therefore, various precautions were considered in order to avoid the generation of excessive heat when combining these two compounds. Cooling the putrescine or butyric acid in a freezer before mixing was attempted but problems were experienced with contamination caused by moisture condensation. The more effective approach leveraged the high volatility of the butyric acid. Two vials, containing portions of the separate compounds, were placed in a large jar. Over time, crystals were observed to grow near the top of the vial containing the putrescine. The crystal growth proceeded to the point of forming a plug of solids well above the putrescine level present in the vial. This blocked access of the butyric acid to the unreacted putrescine. Therefore, it was necessary to intermittently puncture these plugs using a glass rod in order for the reaction to proceed. After six weeks, the crystals deposited near the top of the putrescine vials were harvested. They were placed on top of filter paper in Petri dishes and stored in a desiccator. This proved necessary as the crystals were highly deliquescent.

2.2.2. Material characterisation

Elemental analysis was performed on an Elementar Unicube CHNS. The sample size was 5 mg and it was used to ensure sufficient oxygen dosing to ensure complete combustion. Acetanilide and sulfanilamide were used as the calibration standards. All samples were run in triplicate. This included the crystals harvested from the mixtures of butyric acid to putrescine in mole ratios of 2:1, 3:1 and 3.5:1.

Small-angle powder X-ray diffraction (pXRD) was performed using a PANalytical Aeris powder diffractometer with a PIXcel detector wash, with fixed divergence- and receiving slits with Fe-filtered Co-Kα radiation ($\lambda = 1.789$ Å). Indexing and unit cell determination of powder diffraction data was done by BRUKER TOPAS software [10]. The data was indexed on a monoclinic unit cell P21/n (14) selected with the highest goodness-of-fit. Evaluation of the top ten solutions by Chekcell [11] confirmed P21/n as the best space group (SG) for the analysed diffraction data. All initially selected 69 reflections in angular range 4–75° 2θ were successfully indexed together with additional theoretical lines, i.e., lines of low intensities were added in the process.

Fourier transform infrared-attenuated total reflectance (FTIR-ATR) spectra were recorded using a Perkin Elmer Spectrum 100 instrument fitted with an ATR attachment. The wavenumber resolution was 4 cm^{-1} and the average of 16 scans, obtained over the wave number range 650 to 4000 cm^{-1}, are reported.

Both thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were performed simultaneously on a TA Instruments SDT Q600. The TGA set-up employed in this study comprised a 90 μL cylindrical alumina sample cup with the inert purge gas sweeping over the top as shown in the Fig. 1. Nitrogen, flowing at a rate of 80 mL min^{-1}, was used as the purge gas. The scan rate was set at 2 K min^{-1}. Sample mass used was either ca. 5 mg or 10 mg.

2.3. Field trial

For the field trial, 49 trap were used of which nine traps were loaded with the Questlure capsules. For the forty other traps, the sponges were removed from the capsules and replaced with a sponge containing one of the following attractants: neat butyric acid, neat putrescine, or mixtures
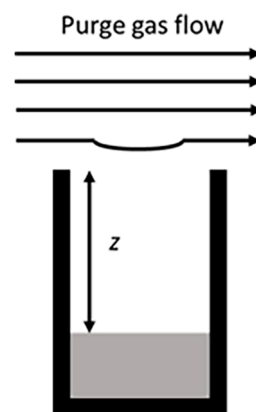

Fig. 1. Schematic showing the sample pan with the nitrogen purge gas flow configurations used for measuring the volatilisation of compounds in the TGA apparatus.

of butyric acid:putrescine at mole ratios of 1:1 and 3:1. A total of 2 g of attractant was pipetted onto the sponges a day before transporting them to the farm. All traps were also loaded with a dichlorvos insecticide pastille located in the lid. An orange orchard (25°42′15.5″S 27°43′35.0″E) was used for the field trials. The traps were placed in a staggered pattern with one trap in each row of trees. Weekly trap collections were made over a period of six weeks.

3. Results and discussion

3.1. Characterisation

Fig. 2 shows the X-ray diffractograms obtained for putrescine and the complex formed by its reaction with butyric acid. The reflections seen for the two compounds clearly differ indicating that a new phase was formed. Actually, all the crystals obtained by reacting the two precursors in different ratios featured the same X-ray diffractogram. This indicates a strong tendency to form one particular complex. The unit cell parameters of this complex were refined to that given in Table 1. It should be noted that these provisional values do not correspond to a structure determination. They simply represent the refinement of the raw pXRD data for indexing and unit cell determination. Single crystal data would be required to perform a full structure determination.

The stoichiometry of the complex was established by elemental analysis. The expected C/N ratios for butyric acid to putrescine mole
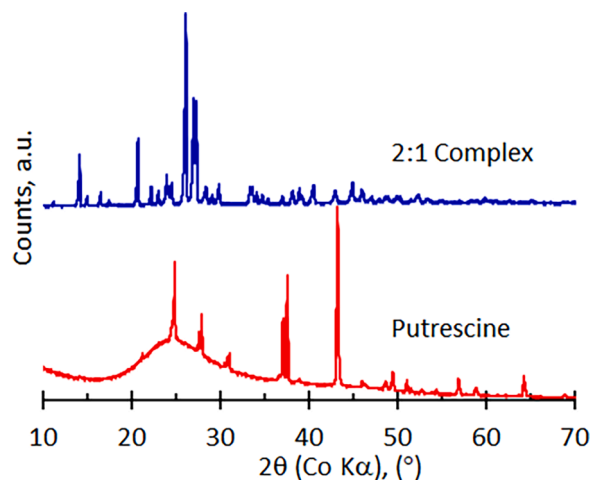

Fig. 2. X-ray diffractograms recorded for putrescine and its complex with butyric acid.

Table 1

Provisional unit cell parameters for the putrescine–butyric acid complex compared to literature results for butyric acid and putrescine.

Parameter	Units	Butyric acid	Putrescine	Complex
Space group	Units	C 2/m (12)	*P b c a* (61)	P2₁/n
A	Å	8.010	6.5516 (13)	20.186 (2)
B	Å	6.820	5.7382(13)	14.577 (2)
C	Å	10.140	14.528(3)	9.984 (1)
A	°	90	90	90
B	°	111.45	90	115.731 (5)
Γ	°	90	90	90
V	Å³	515.563	546.171	2646.6 (6)
References		CSD_1117370 [12]	CSD_132871 [13]	This study

Fig. 3. FTIR spectra for butyric acid, putrescine and the 2:1 complex of the two precursors.

ratios of 1:1, 2:1 and 3:1, are 3.43, 5.15 and 6.85 respectively. The actual measured C/N ratios for the crystal samples investigated varied from 4.92 to 5.17 with an average of 5.05 ± 0.09. The implication is that the crystals obtained represented a 2:1 butyric acid–putrescine complex. Scheme I shows a tentative salt-forming reaction that leads to a structure corresponding to a 2:1 complex.

Fig. 3 compares the Fourier transform infrared spectra for the precursor and the 2:1 complex in the wavenumber range 800 cm⁻¹– 3600 cm⁻¹. For the pure butyric acid, a strong characteristic band at 1 705 cm⁻¹ is assigned to the C=O stretch in dimerised carboxylic acid functional groups. [14]. The putrescine exhibits strong absorption bands at 3 328 cm⁻¹ (–NH₂ stretch of aliphatic primary amines), 1 635 cm⁻¹ (NH bend), and 1 050 cm⁻¹ (CN stretch). Noteworthy is the absence, in the 2:1 complex, of the free amine band characteristic of the neat putrescine (located at 3 328 cm⁻¹) and carboxylic acid dimer band in butyric acid (located at 1 705 cm⁻¹). The absence of these absorption bands is consistent with the protonation of the amine nitrogen atoms present in the putrescine, i.e. the formation of amine carboxylates as shown in Scheme I. The 2:1 nature of the complex probably results from the fact that putrescine features two primary amine functional groups, each of which can associate with a carboxylic acid group as is present in butyric acid.

3.2. Thermal analysis

Fig. 4 shows representative TGA and DSC scans for the samples investigated. In the present study, benzoic acid was used as a reference compound as it is known to sublimate and because it melts at approximately the same temperature as the butyric acid-putrescine amine carboxylate [15]. Table 2 lists the temperatures at which different mass loss percentages were recorded. These results and the mass loss curves in Fig. 4a indicate that the complex formed by the reaction of butyric acid and putrescine is significantly less volatile than the precursors.

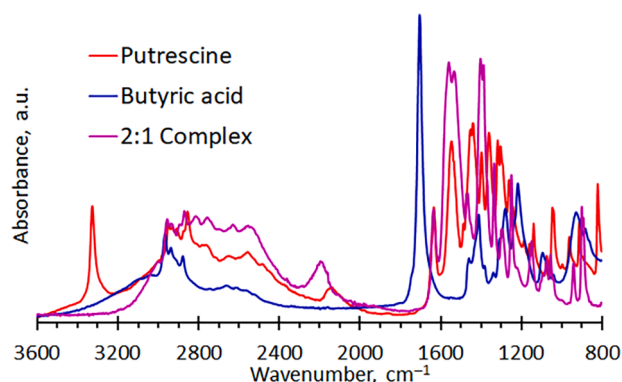Fig. 4b shows the DSC traces obtained for benzoic acid and the 2:1 complex. In each case, the first peak corresponds to a melting endotherm. The complex melts at a slightly higher temperature than benzoic acid. The onset temperatures were determined to be 123.2 ± 0.4 °C and 121.49 ± 0.02 °C for the 2:1 complex and benzoic acid respectively. The corresponding peak temperatures were 124.3 ± 0.2 °C and 122.5 ± 0.1 °C. Unfortunately, it was not possible to calibrate the DSC heat flow response in the temperature range where these two substances melted. However, an estimate of the melting enthalpy was obtained by scaling the measured heat release of the 2:1 complex with that found for the benzoic acid. Applying the literature value of 17.4 kJ mol⁻¹ for the melting enthalpy of benzoic acid [15] led to an estimate of 160 ± 6 J g⁻¹ for the 2:1 complex. The second peak, seen in each of the two DSC traces, reflects the progress of the endothermic evaporation of the samples.

In the experimental set-up shown in Fig. 1, the rate of volatilisation (sublimation or evaporation) of the sample (denoted by the subscript A) is controlled by diffusion through the stagnant gas layer above the sample. If both the vaporised substance and the gas behave ideally, the rate of mass loss is described by Eq. (1) [16]:

$$\Phi_A = \frac{M_A D_A P_A}{RT} = \frac{z}{A} \frac{dm_A}{dt} \tag{1}$$

where Φ_A is the gas permeability of the evaporating substance in g m⁻¹s⁻¹; M_A (g mol⁻¹) is the effective molar mass of the vaporising compound; D_A (m²s⁻¹) is the diffusion coefficient; P_A (Pa) is the sample vapour pressure at temperature T (K); $R = 8.3145$ J mol⁻¹K⁻¹ is the gas constant; A (m²) is the cross-sectional surface area of the sample cup, and z (m) the depth of the gas-filled part of the sample cup, and dm_A/dt is the TGA-measured mass loss rate (i.e. sublimation/evaporation rate) in g s⁻¹.
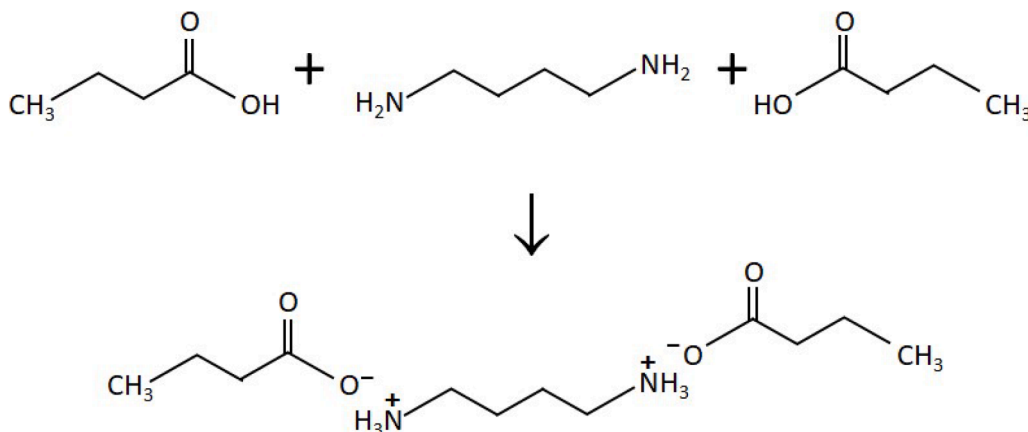
Scheme I. Tentative salt-forming reaction leading to the formation of a dicarboxylate corresponding to a 2:1 complex of butyric acid and putrescine.
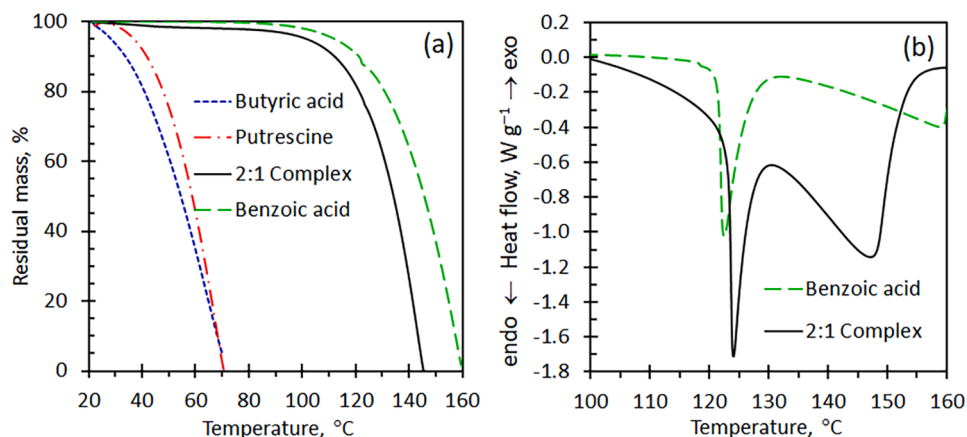
Fig. 4. (a) Thermogravimetric and (b) differential scanning calorimetry results obtained at a scan rate of 2 °C min^{-1}.

Table 2
Summary of TGA mass loss results for sample size of 5 mg.

Temperature (°C) at:	Butyric acid	Putrescine	Complex	Benzoic acid
10% mass loss	33.5	41.8	111.7	121.7
50% mass loss	54.1	58.8	134.0	146.0
90% mass loss	68.1	68.1	143.4	157.7

Note that the right-hand side of Eq. (1) corresponds to measured mass loss rates determined by the geometry of the system and the pressure and temperature imposed on it. In contrast, Φ_A is entirely a material property that links the diffusional mass flux of the substance to the thickness of the quiescent gas layer. While Φ_A is a strong function of temperature, it varies only weakly with pressure as the diffusion coefficient is affected by the latter. At near ambient conditions, the vapour pressure of a substance can often be represented by an Antoine-type equation:

$$P_A = \exp\left[a - \frac{b}{T+c}\right] \qquad (2)$$

where a, b and c are the Antoine vapour pressure constants.

The diffusion coefficient in gas G can be estimated using the Fuller correlation [17]:

$$D_A = \left[\frac{10^{-7}\sqrt{1/M_A + 1/M_G}}{P\left(\sqrt[3]{V_A}+\sqrt[3]{V_G}\right)^2}\right]T^{1.75} \qquad (3)$$

where P is absolute system pressure measured in atmosphere, M_A and M_G are the effective molar masses of the sample and the gas G respectively, while V_A and V_G refer to the corresponding diffusion volumes. Since these values are invariant, they can be combined into a constant C_A. Combining the expressions for the vapour pressure and the diffusion coefficient, one obtains the following temperature dependence for the gas permeability:

$$\Phi_A = \frac{M_A C_A T^{3/4}}{R}\exp\left[a - \frac{b}{T+c}\right] \qquad (4)$$

In the TGA experimental setup, values of Φ_A at different temperatures can be estimated from the mass loss rate (dm_A/dt):

$$\Phi_A = \frac{z_o\left[1 - m_A/(\rho_A V_p)\right]}{A}\frac{dm_A}{dt} \qquad (5)$$

where z_o refers to the initial height of the air in the pan, A is the cross-sectional area of the sample pan, m_A is the time-dependent sample mass in the pan, ρ_A refers to the density of the sample and V_p is the empty volume of the pan. Furthermore, the term in square brackets links the

gas-filled space in the cup to the amount of substance located at the bottom. Ideally, the TGA experiments are conducted under isothermal conditions. However, Eq. (5) can also be applied when conducting dynamic temperature runs at low scan rates [18].

The validity of the experimental approach was examined using benzoic acid. To achieve this, values of Φ_A calculated from the TGA measured mass loss rates, using Eq. (5) were compared to values calculated using Eq. (4). The information listed in Table 3 enabled the latter approach. Fig. 5a compares the estimates based on the experimental TGA mass loss rates to the Φ_A values predicted on the basis of the physical properties of benzoic acid. The agreement is excellent in the evaporation region but, on average, the experimental values are more than 10 % lower in the sublimation range. This is fair considering the simplifying assumptions on which Eq. (5) is based.

Fig. 5b shows experimental Φ_A values and least-squares data fits of Eq. (4). Table 4 lists the regressed Antoine coefficients obtained for putrescine and the 2:1 complex (Eq. (2)). It was not possible to obtain reproducible mass loss rate data for butyric acid. However, the theoretical Φ_A curve for this substance is shown in Fig. 5b based on the temperature dependence of the vapour pressure and the diffusion coefficient listed in Table 3. The important message, conveyed by the curves in Fig. 5b, is that the volatility of the 2:1 complex is much lower than that of the butyric acid and putrescine precursors.

3.3. Field trial

Fig. 6 shows a summary of the field trial results. The cumulative number of flies and non-target insects and spiders are shown plotted as a function of time. Lure A, based on neat butyric acid, and Lure D (the 3:1

Table 3
Material constants for the calculation of vapour pressures and diffusion coefficients.

Parameter (Symbol)	Units	Benzoic acid	Butyric acid	Nitrogen
Molar mass (M_A)	g mol^{-1}	122.12	88.11	28.013
Diffusion volume (V_A)	cm^3 mol^{-1}	119.08	94.3	18.5
Diffusion constant (C_A)	m^{2}s^{-1}K$^{-1.75}$	4.31×10^{-10}	4.93×10^{-10}	–
Antoine (evaporation)		NIST*	[19]	
a		10.3118	21.741	–
b		4078.7	3532.9	–
c		−127.484	−92.99	–
Antoine (sublimation)		[20]		
a		26.633		
b		6396.9		
c		−77.05		

* NIST: https://webbook.nist.gov/cgi/cbook.cgi?ID=C65850&mask=4#Thermo-Phase.
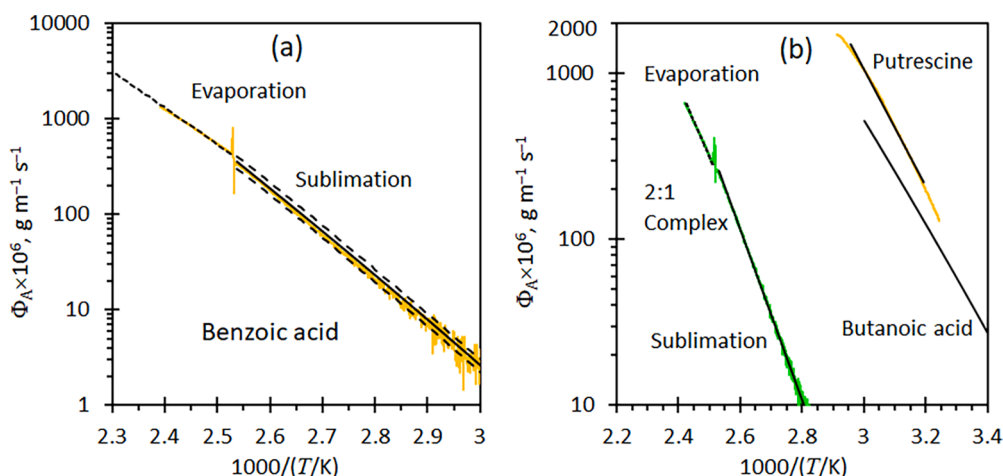
Fig. 5. (a) Experimental (coloured) and predicted (black lines) gas phase permeability for benzoic acid. The dashed lines in the sublimation range represent 15% error margins. (b) Experimental Φ_A values from TGA mass loss rates. The solid black lines show data fits based on Eq. (4).

Table 4
Estimated data for the vapour pressures and diffusion coefficients of putrescine and its complex with butyric acid. Vapour pressure parameters were obtained using least squares regression of the TGA data.

Parameter (Symbol)	Units	Putrescine	2:1 complex
Molar mass (M_A)	g mol^{-1}	88.15	257.37
Diffusion volume (V_A)	cm^3 mol^{-1}	151.08	–
Diffusion constant (C_A)*	m^{2}s^{-1}K$^{-1.75}$	4.02×10^{-10}	4.62×10^{-10}
Antoine (evaporation)		(40–65 °C)	(80–122 °C)
a		26.95	23.96
b		7490.1	8396.9
c		0	0
Antoine (sublimation)			(125–150 °C)
a			31.09
b			11,188
c			0

* The values of C_A for the 2:1 complex was arbitrarily set equal to a mole fraction weighted average over the values for the two precursor compounds.

butyric acid to putrescine mixture) performed worst with only about forty flies caught in ten traps over a period of six weeks. A similar number of non-target creatures was caught in the traps containing these lures and this also applied to Lure E, the commercial product used as reference. Lure B, the neat putrescine, proved to be the most effective attractant for flies and it was also least so for the other bugs. The second-best performance was achieved with Lure C, the 1:1 mixture. It also captured the second lowest number of non-target insects plus spiders. A rationalisation of these results is possible if it is hypothesised that the putrescine is the better fly attractant and that it has a repellent action on the non-flies. The amine carboxylate contains two butyric acid moieties to one of putrescine. Therefore, the better performance of Lure C may be explained by the fact that this combination had some free putrescine. Mixture D contained an excess of butyric acid and this apparently resulted in a lower volatility form rendering the putrescine ineffective.

4. Conclusions

Butyric acid and putrescine were mixed in various mole ratios. Irrespective of the stoichiometry used, a particular crystalline amine carboxylate was obtained. It corresponded to two butyric acid molecules for each putrescine. The resulting amine carboxylate was found to melt at 123.2 ± 0.4 °C. The enthalpy of melting was estimated to be about 160 ± 6 J g^{-1}. Dynamic thermogravimetric analysis was used to study the sublimation rate of this complex relative to benzoic acid as the reference compound. During this process, a new material property was identified that provides an indicative measure of the volatility of a substance in a quiescent gas, e.g. air.

A preliminary field trial in an orange orchard showed neat putrescine to be more effective as a fly attractant than neat butyric acid and
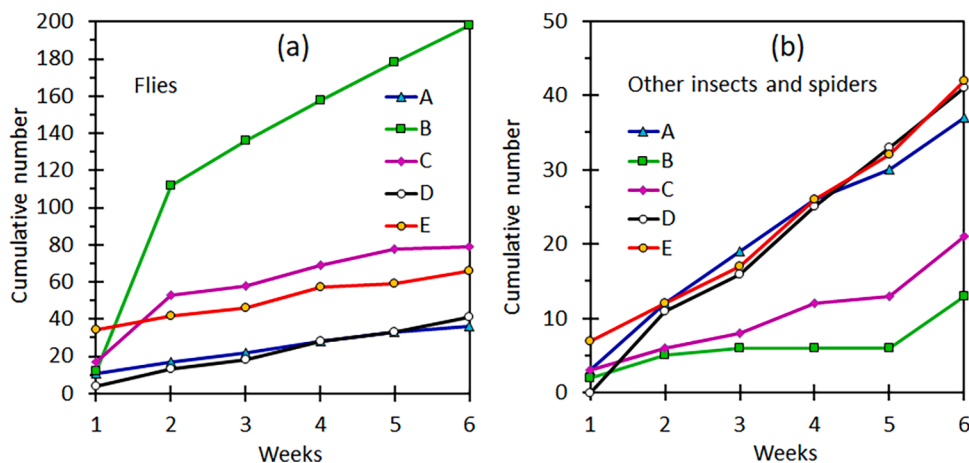

Fig. 6. Cumulative number of (a) flies and (b) non-target insects and spiders caught in the traps over a period of six weeks. The lure compositions, in terms of the butyric acid to putrescine mole ratios, was as follows: $A = 1:0$; $B = 0:1$; $C = 1:1$; $D = 3:1$; and $E =$ hydrolysed protein used as reference.

mixtures with the latter. Adding butyric acid significantly decreased the lure properties of putrescine. This is tentatively attributed to an excessive reduction in the volatility of the putrescine caused by the salt formation. Nonetheless this work proves that complex formation of active ingredients in fly lures has the potential to reduce the volatility and increase the longevity of each application—provided that measures can be found to optimise effectiveness with release rate behaviour.

Consent to participate

Consent to participate was not necessary as human subjects were not involved in any experiments

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors did not use any AI or AI-assisted technologies at all.

Ethical approval

Ethical clearance to conduct the field trial was obtained from the Faculty of Health Sciences ethics committee of the University of Pretoria, protocol number (EBIT/207/2020).

CRediT authorship contribution statement

Franco Pretorius: Writing – review & editing, Writing – original draft, Validation, Software, Resources, Methodology, Formal analysis, Data curation. **Elizabeth L. du Toit:** Writing – review & editing, Supervision. **Isbé van der Westhuizen:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Maria T. Atanasova:** Software, Formal analysis. **Walter W Focke:** Conceptualization, Methodology, Software, Validation, Formal analysis, Resources, Data curation, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Walter Focke reports financial support was provided by Paper Manufacturers Association of South Africa. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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